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Proceedings of the 21st Annual Aquatic Toxicity Workshop: October 3-5, 1994, Sarnia, Ontario

Comptes rendus du 21eme atelier annuel sur la toxicité aquatique: du 3 au 5 octobre 1994, Sarnia, Ontario

Editors/Éditeurs

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Canadian Technical Report of Fisheries and Aquatic Sciences 2050

Rapport technique canadien des sciences halieutiques et aquatiques 2050

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PREFACE/PREFACE

The 21st Annual Aquatic Toxicity Workshop was held at the Holiday Inn in Sarnia, Ontario, October 3 to 5, 1994. The Workshop included 2 plenary presentations, 85 platform presentations and 46 papers in poster sessions. Total attendance was 258.

The 21st Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the proceedings are published with the support of the Department of Fisheries and Oceans.

Le 21ème atelier annuel sur la toxicité a eu lieu le Holiday Inn, Sarnia, Ontario les 3 au 5 octobre 1994. Le atelier a donné lieu a 2 communications lors de séances plénières, 85 exposés d'invités d'honneur 46 communications par affichage. 258 personnes ont assisté au atelier.

Le 21ème atelier annuel sur la toxicité aquatique a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS/REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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PLENARY SESSION/SÉANCE PLÉNIÈRE

CONTAMINANTS IN AND THEIR EFFECTS ON GREAT LAKES BIRDS

John P. Giesy
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI

AN INDUSTRIAL PERSPECTIVE

Michael Ashar
Sunoco, Inc.
Sarnia, ON

PLATFORM PRESENTATIONS/SÉANCES EXPOSÉS

REPRODUCTIVE TOXICOLOGY
SESSION CHAIR: G.J. Van Der Kraak

ENDOCRINE RESPONSES TO CONTAMINANTS IN TELEOST FISH - ROLE OF CORTISOL AND THYROID HORMONES. A. Hontela. Département de Sciences Biologiques, TOXEN, Université du Québec à Montréal, Montréal, PQ, H3C 3P8.

Toxic chemicals present in the aquatic environment challenge the ability of fish to maintain physiological homeostasis. At sublethal doses, homeostasis may be maintained, in part through increased regulatory activity mediated by the endocrine system. Cortisol and thyroid hormones, secreted by the interrenal and the thyroid gland respectively, regulate metabolism and have effects on reproduction, growth, osmoregulation and the immune function. Several environmental contaminants alter plasma levels of cortisol or the thyroid hormones following acute exposures. However, our understanding of the effects of chronic exposures on these endocrine systems, of the physiological consequences of the potential endocrine disruptions caused by the toxic chemicals and of the repercussions of these physiological effects at the level of fish communities and populations, is limited. We have investigated the role of cortisol and of thyroid hormones in the physiological response to contaminants in several teleosts (*Perca flavescens, Esox lucius, Oncorhynchus mykiss, Catostomus commersoni*) exposed to sublethal doses of contaminants (heavy metals, organic contaminants) in the laboratory or in the field at selected contaminated and reference sites. Fish were captured by standardized methods and blood, endocrine tissues, liver and gonads were sampled. Blood hormone levels were assayed with RIA, standard methods were used to assay biochemical parameters such as blood glucose or liver glycogen, morphological changes were quantified with an Image Analyser. Exposure to contaminants alters plasma cortisol and thyroid hormone levels, as well as blood glucose and liver glycogen. Studies investigating the link between these physiological responses and effects on somatic and gonadal growth in fish exposed to environmental contaminants are ongoing.

EXAMINATION OF REPRODUCTIVE DYSFUNCTION IN FISH THROUGH SHORT-TERM EXPOSURE OF MATURE GOLDFISH TO CONTAMINANTS IN A FIELD AND LABORATORY SETTING. M.E. McMaster[1], G.J. Van Der Kraak[1] and K.R. Munkittrick[2]. [1]Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1; [2]GLLFAS, Department of Fisheries and Oceans, Burlington, ON, L7R 4A6.

Changes in reproductive fitness were monitored in goldfish exposed to bleached kraft pulp mill effluent (BKME) both *in situ* and in the laboratory. *In vitro* production of the dominant reproductive steroids (testosterone and 11-detotestosterone, males; testosterone and 17ß-estradiol, females) were reduced within four days of effluent exposure in situ. Similar exposures using effluent transported from the field to the laboratory had little effect following a four day exposure but a marked inhibitory effect on steroid production after eight days. Similar lab tests using effluent collected three and six months later following several changes in mill processes, failed to demonstrate similar impairment of the steroid biosynthetic capacity of gonadal tissues. This recovery in steroid production was confirmed by in situ exposures conducted in May, 1994. These studies have identified a short-term exposure protocol for measuring reprodutive fitness in native fish. We are currently analysing field samples to evaluate whether native fish are exhibiting similar recovery in reproductive fitness.

DECREASED REPRODUCTIVE FITNESS IN GOLDFISH EXPOSED TO THE PLANT STEROL ß-SITOSTEROL. G. Van Der Kraak and D.L. MacLatchy. Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1.

The effects of ß-sitosterol (SIT), a primary plant sterol present in bleached kraft mill effluent, on reproductive performance in the goldfish was examined. Intraperitoneal injection of SIT into goldfish at doses of 10, 20 or 100 µg/g caused a dose dependent decrease in plasma levels of testosterone and 11-ketotestosterone in males and testosterone and 17ß-estradiol in females. SIT had no effect on plasma gonadotropin levels. Male goldfish exposed to SIT in the water (75-1200 µg/L) in a static system for 12 days or less had reduced levels of plasma testosterone and 11-ketotestosterone. These fish also exhibit decreased in vitro production of testosterone and pregnenolone (the first steroid after cholesterol in the steroidogenic pathway). This suggests that the gonad rather than the pituitary represents the primary target of SIT action. The waterborne exposure experiments show that SIT in the water at dosages found in the vicinity of pulp mills in Ontario can enter goldfish and consequently have reproductive effects.

THE ACUTE LETHALITY TO RAINBOW TROUT OF WATER CONTAMINATED BY AN AUTOMOBILE TIRE. S.G. Abernethy. Aquatic Toxicology Section, Ontario Ministry of Environment and Energy, 125 Resources Road, Etobicoke, ON, M9P 3V6.

Scrap automobile tires have been used to build aquatic structures like artificial reefs and floating breakwalls. Laboratory studies report that waters with submersed tires cause acute lethality to rainbow trout. Since the "tire water" toxicant has not been identified, further tests were conducted for a Toxicity Identification and Reduction Evaluation. Tire water was prepared by submersing a whole scrap tire in 300 litres of water for 10 days. No lethality was observed in static acute tests of fathead minnows and two daphnid species, but 100 percent mortality occurred within 48 hours in trout tests. Similar effects were observed when tire water was prepared with scrap tires collected from an artificial reef in Lake Erie. The tire water was not detoxified by storage, light, aeration, pH adjustment, an anti-oxidant and a metal chelator, but activated carbon did remove the toxicity. The tire water toxicant was not identified by target chemical analyses, but gas chromatography-mass spectrometry scans showed that alkylphenols and ayrlamines were prevalent. Acute lethal and chronic sublethal toxicity to *Daphnia magna* and *Ceriodaphnia dubia* was observed when water was contaminated by rubber particles made from scrap tires. The toxicity of the tire particles was not "exhausted" by repeated contact with water.

SUB-LETHAL EFFECTS IN PULP AND PAPER EFFLUENTS AND RECEIVING WATERS. M.O. Farkas, S.R. Megraw and V.A. Naish. Domtar Research Centre, Senneville, QC, H9X 3L7.

The Pulp and Paper Effluent Regulations under the *Fisheries Act* were amended in 1992 to include testing of mill effluents for sub-lethality. The objective is to determine the potential for sub-lethal effects of effluents in the receiving environment. We examined the sub-lethality of several pulp and paper mill effluents and their receiving waters using the *Ceriodaphnia dubia* survival and reproductive test. The results indicate that laboratory bioassays of effluents do not accurately reflect the degree of toxicity measured in the receiving environment. This paper discusses the relationship between effluent and receiving water measurements of sub-lethality and describes some of the factors which can influence the *Ceriodaphnia* test results.

TOXICITY OF INDUSTRIAL EFFLUENTS
SESSION CHAIR: S.G. ABERNETHY

FATE OF HYDROCARBONS IN *Pseudopleuronectes americanus* EXPOSED TO HIBERNIA CRUDE OIL.

J. Hellou and C. Upshall. Department of Fisheries and Oceans, St. Johns, NF, A1C 5X1.

ABSTRACT

Winter flounder were exposed to different concentrations of Hibernia crude, for four months during the winter. Concentrations of oil in sediments ranged from 0.09 to 4.5 mg/g (dry weight), at the beginning, to 0.10 to 0.90 mg/g at the termination of the experiment. At the end of the exposure, muscle, liver and gall bladder bile were analysed for different types of hydrocarbons using a multispectroscopic approach (uv/f and GC-MS techniques). A dose-response was observed in muscle when analysing for the presence of free hydrocarbons, either as total unsaturated or 27 parental and alkylated PAC. A dose-response was also observed for the gall bladder bile metabolites analysed as glucuronide and sulfate conjugates. The threshold concentration of hydrocarbons in sediments, above which statisticaly significant chemical accumulation or elimination of aromatic compounds was observed is presented in terms of various hydrocarbon components in sediments. The preferential bioaccumulation of monocyclic aromatic hydrocarbons belonging to the unresolved complex mixture (UCM) of the oil over that of parental and alkylated PAH detected in muscle was apparent. The predominance of naphthalene and phenanthrene metabolites in the resolved components of the metabolites mixture over that of the resolved lower molecular weight alkylbenzene derivatives was also observed.

INTRODUCTION

The Grand Banks of the Northwest Atlantic, east of the island of Newfoundland are slatted for petroleum development in the near future. The general chemical properties and acute toxicity of Hibernia crude oil collected during the exploration of the fields has been previously reported in the literature (MacLean, 1988).

The present experiment involved exposing male winter flounder, *Pseudopleuronectes americanus*, to five concentrations of oil in sediments, ranging from a control site to realistically higher levels (10-50 times, depending on components analysed). Exposure was performed during the winter, a period when flounder are in a dormant state and naturally fasting (Fletcher 1977, Fletcher and King 1978). Feeding has been shown to increase metabolism of xenobiotics and reduce bioaccumulation. The water temperature was typical of that found in many regions of the Northwest Atlantic (-1 to +30°C). A higher temperature is generally correlated with a higher water solubility of hydrocarbons, but also to higher metabolic rates and therefore lower bioaccumulation in fish. The grain size (>65 µm) and total organic content (0.8%) of the sediments was typical of nearshore areas where the spill would concentrate. Hydrocarbons are hydrophobic and lipophilic and tend to accumulate on smaller particulates, richer in organic material. A higher organic content translates into a lower bioavailability of contaminants. Fish were all males, because available in larger number and this sex eliminates the variable related to spawning in females that could also affect uptake and elimination rates. Fish were all of a similar size to eliminate age as another possible factor influencing the fate (possibly lower metabolism). The tissues chosen for this study are representative of: (1) long-term accumulation: muscle; (2) short-term accumulation: liver; and (3) short-term elimination: gall bladder bile.

This study was undertaken to determine the fate of Hibernia crude oil in flatfish and to correlate the various approaches available to determine exposure. In overall terms, the present exposure represents an extreme case of bioavailability and response of flatfish.

MATERIALS AND METHODS

Male winter flounder were placed in six tanks (300 L) containing sediments (45 kg) obtained from a remote area. Mean fish weight and length, per treatment ranged from 243 to 280 g and 27 to 29 cm. One tank containing the sediments was used as a control, while 5, 25, 50, 100 and 250 ml of oil were added to sediments in five other tanks, prior to the addition of seawater and fish a week later. The fish and experimental tanks are referred to as E-5, 25, 50, 100 and 250, for simplification. Sediments were subsampled from each tank, at the begining and end of the four months exposure and analysed for total unsaturates, a series of 27 PAC and for other PAH (Hellou et al. 1994). Hibernia crude was separated by chromatography and the non-volatile components analysed using a multispectroscopic approach (Hellou et al. 1994). Muscle, liver and gall bladder bile metabolites obtained from fish sacrificed at the end of the experiment, were also analysed by uv/f and GC-MS, (Hellou et al. 1994, Hellou and Upshall 1994).

RESULTS

The present sample of Hibernia crude oil had a density of 0.813 g/ml, the non-volatile unsaturated hydrocarbons represented 32%, while the saturated hydrocarbons represented 42% of the oil. Synchronous fluorescence ($\Delta\lambda$=25nm) of the oil, water soluble fraction and sediments extract displayed a maximum at 280/305nm and minor ones at 335/360 and 390/415 nm (Fig. 1). The higher intensity of the first maxima indicates that monocyclic aromatic compounds predominate over 2, 3, 4, 5 and 6-ring species (Wakeham 1977, Vo-Dinh 1978, Baudot et al. 1991). GC-MS analysis of the fractions indicated the presence of resolved components, easily identified in both the saturated and unsaturated hydrocarbon fractions (Hellou et al. 1994).

Extraction, chromatography and analysis of the sediments at the end of the four months exposure indicated that the resolved aromatic and n-alkane hydrocarbons were reduced in abundance in the weathered oil, while the more persistant alkylbenzenes and other more branched, isoprenoid or cyclic hydrocarbons were more predominant compared to the fresh oil (Hellou et al. 1994). Concentration of specific PAC in sediment extracts is presented in Fig. 2.

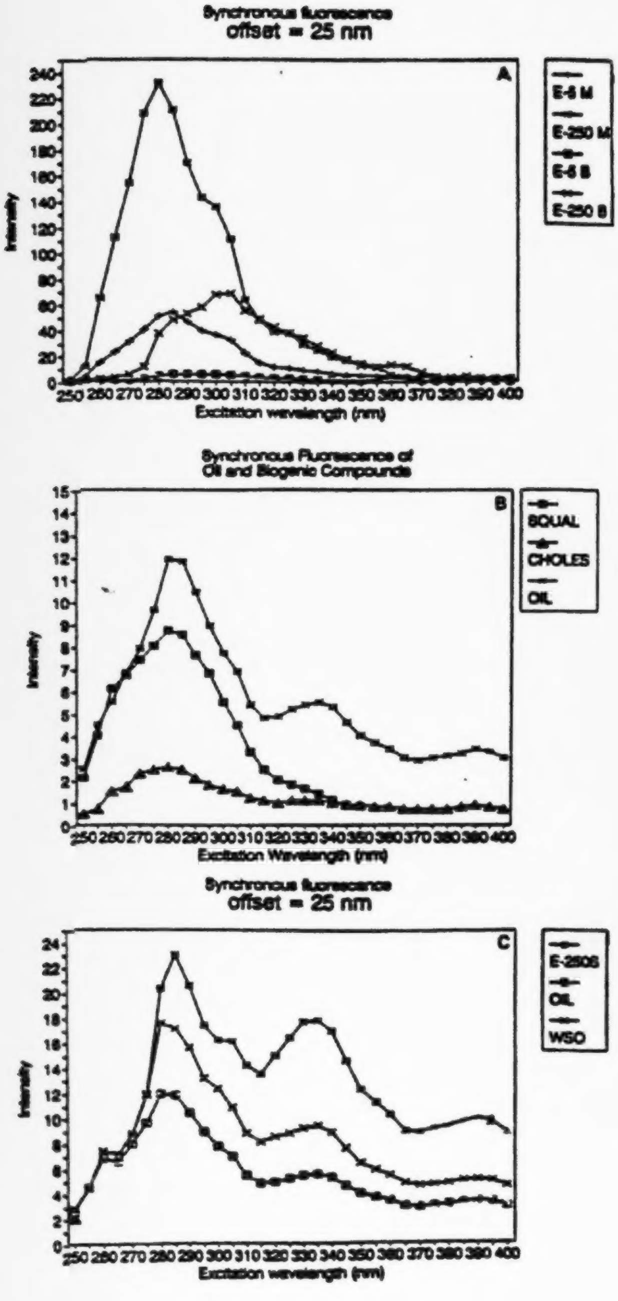
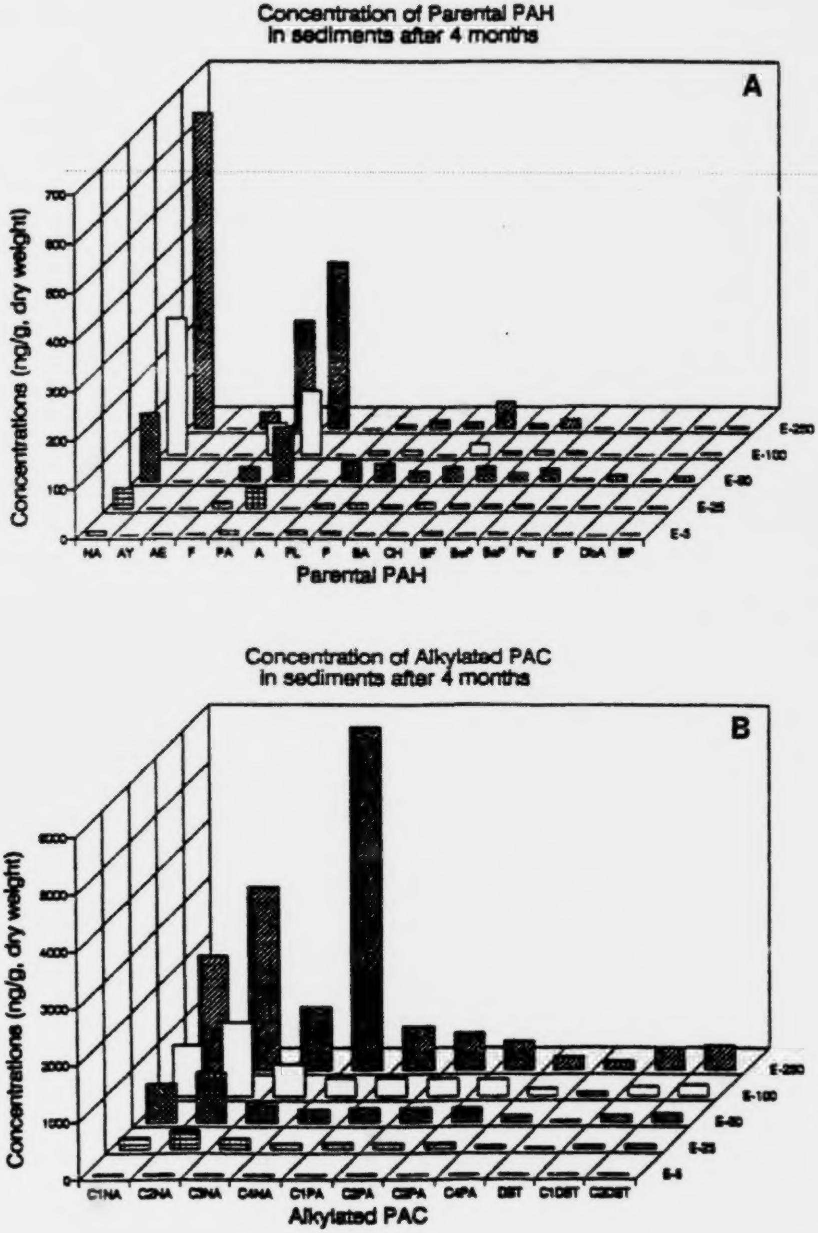


Fig. 1. Results of synchronous fluorescence analyses. A - Analysis of muscle (M) and bile (B) from lowest (E-5) and highest (E-250) exposures. B - Analysis of squalene, cholesterol and Hibernia crude oil. C - Analysis of sediments extract (E-250S), Hibernia crude oil (oil) and the water soluble fraction of the oil (WSO).


Concentration of Parental PAH in sediments after 4 months

Concentration of Alkylated PAC in sediments after 4 months

Fig. 2. Concentration of aromatic hydrocarbons in sediments. A-Concentrations of the parental PAH, at the 5 exposures. B-Concentrations of the alkylated PAH and DBT derivatives, at the 5 exposures.

Liver tissue obtained from fish in tanks E-250, E-100 and E-5 and muscle obtained from all exposures were analysed by uv/f and/or GC-MS (Table 1). GC-MS-SIM allowed the detection and quantification of 27 PAC (parental, alkylated and S heterocycles, Table 2). As well, muscle was analysed by GC-MS-TIC, to investigate the presence of other free hydrocarbons in the extracts. The gall bladder bile of fish obtained from all exposures was analysed for glucuronide and sulfate metabolites, using enzymatic hydrolysis, followed by uv/f and SF analysis, acetylation of the alcohols and analysis by GC-MS (Hellou and Upshall 1994).

Table 1. Correlation of hydrocarbon concentrations[1] observed in sediments, muscle, bile and liver samples of *Pseudopleuronectes americanus*.

Sample	Sediments		Muscle	Muscle	Bile	Liver
origin	Σ27 PAC[2]	uv/f[3]	Σ27 PAC[2]	uv/f	uv/f	uv/f
	(mg/g) end of 4 months		(µg/g) mean	(µg/g) mean	(µg/µl) mean	(µg/g) mean
Offshore	NA		ND	ND	NA	1.0
Control	0.09	ND	0.02	ND	0.13	NA
E-5	0.10	0.01	0.03	0.01	0.22	2.5
E-5:	threshold concentration for production of bile metabolites[4]					
E-25	0.24	0.03	0.01	0.05	0.62	NA
E-25:	threshold concentration for accumulation of unsaturated hydrocarbons[5]					
E-50	0.65	0.04	0.22	0.09	1.3	NA
E-100	1.7	0.13	0.67	0.12	2.4	3.5
E-250	2.2	0.27	2.5	0.17	3.4	4.3

[1] All concentrations expressed in terms of dry weights, except for bile expressed in terms of µl. [2] Sum of mean PAC concentrations, when present in more than 80% of analysed samples, detailed concentrations reported in Hellou et al. (1994). [3] uv/f results refer to the chrysene standard (310/360 nm). [4] Detailed in Hellou et al. (1994). [5] Detailed in Hellou and Upshall (1994). NA: not available, ND: not detected.

DISCUSSION

A dose-response was observed for hydrocarbons accumulated in muscle tissue, regardless of analytical approach (Table 1). The E-25 level of weathered petroleum in sediments represented the threshold above which hydrocarbons could be detected in muscle tissue (Hellou et al. 1994). Hydrocarbons were detectable in liver at a higher concentration than in muscle. Liver concentrations did not appear to significantly increase with the level of exposure, but were higher than those of offshore fish. The following order of increasing concentrations was observed for groups of free aromatic hydrocarbons in muscle of flounder analysed from the higher exposures:

UCM and alkylbenzenes > alkylated PAH > parental PAH > DBT group

Of the parental PAH, the lower molecular weight species (2 and 3 rings) were more predominant, with mean concentrations increasing as indicated (Table 2):

F > PA > DBT > CH > Fl and P

Alkylated NA, DBT and PA displayed generaly a decreasing accumulation with increasing substitution, for a particular ring. The mean concentration of C-1 and C-2 DBT in tissues was similar to that of C-1 and C-2 PA. Combining all of the above results obtained when analysing specificaly for 27 PAC translates into the following order of bioaccumulation (compounds detected in over 80% of the samples belonging to the three higher exposures):

C-2NA > C-3NA >C-4NA > F > PA>DBT > C-1PA=C-1DBT > C-2PA=C-2DBT >CH

Analysis by uv/f-SF displayed a maxima at 285/310 and 305/330 nm (Fig. 2A). These wavelength pairs are characteristic of monocyclic aromatic compounds (Vo-Dinh 1978, Baudot et al. 1991, Wakeham 1977). Confirmation of the predominance of monocyclic species not analysed by GC-MS-SIM was obtained from the GC-MS-TIC where an unresolved complex mixture was apparent and alkylbenzene components could be extracted from the chromatogram (Hellou et al. 1994).

Table 2. PAH bioaccumulated in flatfish muscle.

PAH[1]	(MW)	Water Solubility[2]	Mean concentration E-50 / E-100 /E-250	BCF[4] E-50 / E-100 /E-250
C-1NA	(142)		32 / 350 / 800	1.2 / 6.3 / 12
C-2NA	(156)		85 / 460 / 1100	1.3 / 2.5 / 4.6
C-3NA	(170)		52 / 150 / 310	1.1 / 0.7 / 1.3
C-4NA	(184)		28 / 86 / 170	0.60 / 0.41 / 0.71
F	(166)	1.83	2.3 / 9 / 33	0.95 / 0.90 / 0.79
PA	(178)	1.28	3.4 / 6 / 27	0.27 / 0.16 / 0.68
DBT	(184)	1.03	1.5 / 5.4 / 22	0.37 / 0.40 / 1.4
C-1PA	(192)		3.4 / 6.5 / 12	0.08 / 0.06 / 0.09
C-1DBT	(198)		1.9 / 3.8 / 19	0.11 / 0.05 / 0.21
C-2PA	(206)		1.5 / 3 / 7.8	0.02 / 0.02 / 0.05
C-2DBT	(212)		1.5 / 2.9 / 8.1	0.04 / 0.02 / 0.06
C-3PA	(220)		1.6 / 1.6 / 1.9	0.01 / 0.01 / 0.01
Fl[3]	(202)	0.24	0.35 / 0.65 / 0.65	0.02 / 0.10 / 0.12
P[3]	(202)	0.14	0.25 / 0.45 / 0.75	0.02 / 0.06 / 0.09
CH	(228)	0.003	0.77 / 0.82 / 1.6	0.06 / 0.03 / 0.07

[1] PAH present in over 80% of the samples collected from the reported exposures. [2] Mean values obtained from Pearlman (1984) in terms of µmole/L and transformed into mg/L, (temperature=25°C). Solubility would be expected 3 times lower at 0°C. [3] PAH were present in 50, 80 and 30% of the samples from the E-50, 100 and 250 exposures, respectively. [4] Derived BCF for E-250 could be questioned in terms of the PAC concentrations obtained in the sediment sample.

The metabolites present in the gall bladder bile also displayed a dose-response. Levels of metabolites were above those of control fish when the exposure was higher than E-5. Analysis by SF displayed the same maxima as in muscle, along with three more maxima of lower intensity at 330/355, 360/385 and 385/410 nm (Fig. 2A). These last three wavelength pairs are characteristic of bicyclic, tricyclic and tetracyclic aromatic compounds (Vo-Dinh 1978, Wakeham 1977, Baudot et al. 1991). The GC-MS-TIC displayed the presence of naphthalene and phenanthrene derivatives overlapping alkylbenzene type compounds that could be extracted from the MS chromatogram (Hellou and Upshall 1994).

The effect of biogenic components present in the extracts, such as squalene in muscle and steroids in the bile, was investigated. Although these isoprenoid derivatives display a maxima at 280/305 nm (Fig. 2B), their concentration did not increase with exposure.

Therefore it appears reasonable to assign the SF maxima observed in tissues to monocyclic aromatic species (Hellou and Upshall 1993, 1994).

As outlined previously (Hellou et al. 1994) parental PAH represent a very minor fraction of the total unsaturated hydrocarbons accumulated. Higher molecular weight PAH (4, 5 and 6 rings), also implicated in carcinogenicity, were proposed by Varanassi and Stein (1991) and Varanasi et al. (1989) to metabolise more readily than lower molecular weight counterparts (2 and 3 rings). Although this was not observed in the bile, possibly due to the low concentrations of metabolites, Table 2 points out that fluoranthene (Fl) and pyrene (P) were detectable in less fish than the other listed parental PAH.

An important effect of petroleum spills has been the presence of volatile hydrocarbons (e.g. benzene, xylene and toluene), causing tainting in fish products (Heil and Lindsay 1990, Connell and Miller 1981). In other cases of environmental contamination, exposure to phenols has been implicated with toxic or beneficial effects in biota and man (Lopp et al. 1992, Hwang et al. 1992). Although the effect of linear alkylbenzene sulfonates, used as surfactants has been previously examined, the effect of the more diverse structural group of alkylbenzenes present in oils has not been addressed. The present four month experiment examining a series of biomarkers (Payne, in preparation) identified an increase in liver lipids and MFO enzymes activity, therefore a molecular and cellular disturbance. More questions regarding the effects on organs, individuals, communities, populations or ecosystems are also raised (Malins 1989, Gray, 1989, Clements and Kiffney 1994).

SUMMARY

The present multispectroscopic approach investigating the fate of hydrocarbons in tissues of flatfish has demonstrated that monocyclic aromatic hydrocarbons, which represent the bulk of most oils accumulate in muscle. Monocyclic aromatics are more resistant to weathering and more water soluble than hydrocarbons with a higher aromaticity and represent a class of compounds that need further investigation in terms of long-term effects. A dose-response was observed in muscle above the E-25 level and in bile above the E-5 level of oil in sediments. Although liver concentrations were higher than in muscle, it is the liver to muscle ratio of concentrations that was indicative of long-term bioaccumulation.

REFERENCES

Baudot, P.H., M.L. Viriot, J.C. Andre, J.Y. Jezequel and M. Lafontaine. 1991. Analysis of polycyclic aromatic hydrocarbons by synchronous fluorescence spectrometry: application to occupational health. Analysis 19: 85-97.

Clement, W.H., and P.M. Kiffney. 1994. Assessing contaminant effects at higher levels of biological organisation. Environ. Toxicol. Chem. 13: 357-359.

Connell, D.W., and G.J. Miller. 1981. Petroleum hydrocarbons in aquatic ecosystems-behavior and effects of sublethal concentrations. Part 2. CRC Crit. Rev. Environ. Control. 11: 105-162.

Fletcher, G.L. 1977. Circannual cycles of blood plasma freezing point and Na and Cl concentrations in winter flounder (*Pseudopluronectes americanus*): correlation with water temperature and photoperiod. Can. J. Zool. 55: 789-795.

Fletcher, G.L., and M. King. 1978. Seasonal dynamics of Cu, Zn, Ca and Mg in gonads and liver of winter flounder (*Pseudopleuronectes americanus*): evidence for summer storage of Zn for winter gonad development in females. Can. J. Zool. 56: 284-290.

Gray, J.S. 1989. Do bioassays adequately predict ecological effects of pollutants? Hydrobiol. 188/189: 397-402.

Heil, T.P. and R.C. Lindsay. 1990. Environmental and industrial factors relating to flavor tainting of fish in the upper Wisconsin River. J. Environ. Sci. Health 25B: 527-552.

Hellou, J. and C. Upshall. 1993. Effect of biogenic impurities on polycyclic aromatic compounds analysis by fluorimetry. J. Environ. Anal. Chem. 53: 249-259.

Hellou, J. and C. Upshall. 1994. Monocyclic aromatic hydrocabons in bile of flounder exposed to a petroleum oil. Inter. J. Environ. Anal. Chem. (In Press).

Hellou, J., C. Upshall, L.L. Fancey and C. Hamilton. 1994. Bioaccumulation of aromatic hydrocarbons from sediments: a dose-response study with flounder (*Pseudopleuronectes americanus*). Arch. Environ. Contam. Toxicol. (In Press).

Hwang, D.F., M.R. Tsai, T. Yoshida and S.S. Jeng. 1992. Toxicity and excretion of butylated hydroxytoluene (BHT) through feed in Japanese eel. Jap. Soc. Sci. Fish. 58: 69-74.

Lopp, A., J.M. Salmon, S. Lahmy, F. Bancel, P. Viallet, U. Kirso and P. Jacquignon. 1992. Modification of benzo(a)pyrene monooxygenase activity by phenolic antioxidants BHA and BHT and their uv-irradiation products in 3Y3 fibroblasts. Polyc. Arom. Com. 3: 29-39.

MacLean, M.M., and K.G. Doe. 1988. The comparative toxicity of crude and refined oils to *Daphnia magna* and *Artemia.* Environmental Program Directorate, Environmental Program Services, Environment Canada.

Malins, D.C. 1989. The use of environmental assays for impact assessment. Hydrobiol. 188/189: 87-91.

Varanasi, U., and J. E. Stein. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environ. Health Perspect. 90: 93-100.

Varanasi, U., J.E. Stein and M. Nishimoto. 1989. Biotransformation and disposition of PAH in fish, p. 93-150. *In*: U. Varanasi, (ed.) Metabolism of polycyclic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, FL.

Vo-Dinh, T. 1978. Multicomponent analysis by synchronous fluorescence spectrometry. Anal. Chem. 50: 396-401.

Wakeham, S.G. 1977. Synchronous fluorescence spectroscopy and its application to indigenous and petroleum-derived hydrocarbons in lacustrine sediments. Environ. Sci. Technol. 11: 272-276.

CASE STUDIES FOR TOXICITY TESTING AND RISK ASSESSMENT RELATED TO THE PLACEMENT OF TEXTILE FACILITIES. R.V. Laughton[1], T. Moran[2] and G. Roy[3]. [1]Pollutech Environmental Limited, 68 Westgate Road, Oakville, ON, L6L 5N2; [2]Pollutech Environmental Limited, 1149 Vanier Road, Sarnia, ON, N7S 3Y6; [3]Republic Consultants Inc., 45-34th Avenue, Edmundston, NB, E3V 2T3.

ABSTRACT

The Province of New Brunswick has been actively participating in the establishment of new textile facilities in northeastern and northwestern New Brunswick. A key component of these programs has been the need for detailed environmental studies dealing with the effluent chemical quality and effluent toxicity. In the first case study in northeastern New Brunswick toxicity testing and wastewater treatment process studies were conducted to identify the toxicity of individual process streams, as well as the final effluent. The approach allowed for adjustment of the wastewater treatment process and wastewater stream segregation to eliminate and treat sources of toxicity in the final effluent. As part of the project, a bioassay facility was constructed at the mill and plant operators trained to conduct their own toxicity testing. In the second case study, an environmental assessment was completed for two proposed mills in northwestern New Brunswick with treated wastewater being discharged to the Madawaska River. Toxicity testing is being conducted to determine the overall environmental impact of individual and collective process streams before and after treatment. Testing is being conducted on treated and untreated wastewater collected from existing textile facilities, located in the USA and Hong Kong, having similar processes. The purpose of this presentation is to show the role of toxicity testing as a valuable assessment tool to support standard chemical analysis in determining the potential environmental impact of effluent discharges.

INTRODUCTION

Pollutech Environmental Limited has been actively working within the Province of New Brunswick, concentrating on environmental studies to assist government agencies with the placement of textile facilities. The work conducted to date has included process evaluations for the design of waste treatment facilities, environmental impact studies under the provincial Environmental Assessment Act, and aquatic habitat studies to investigate the impact of the operations on the natural environment.

A key component of the studies to date has been the application of aquatic toxicity tests to assist the process engineering group in identifying potential problems with the effluent from the textile plants. In the first application at an integrated northeastern textile facility using, the toxicity tests were used to identify toxicity from individual process streams, the total plant effluent and the effluent after various stages of treatment. In the second application in northwestern New Brunswick, toxicity testing was completed on two proposed mills to identify the impact of the process streams, before and after treatment, in combination with other industrial and municipal wastes.

In the cases where the textile had not yet been constructed, it was necessary to collect effluent from similar process operations for the investigations. To achieve this, on-site investigations of the plants in Hong Kong and the United States were conducted, followed by treatability testing and toxicity evaluations.

The studies completed to date have shown that the application of toxicity tests is an important investigative tool for process engineering studies. A toxicity testing program can be used in conjunction with routine chemical analysis and treatability testing to better define the expected environmental impact of proposed industrial facilities.

DISCHARGE REQUIREMENTS: The Province of New Brunswick established effluent criteria on a "case-by-case" basis depending on the point of discharge. In the applications studied to date we have evaluated discharges directly to the Atlantic Ocean, discharge into a tidal basin and discharge to a major inland river. In addition to standard discharge criteria (ie. BOD_5, solids, phosphorus, nitrogen), the province has generally stated that the effluents must meet three prime criteria: (1) the effluent must not impart a colour to the waterway into which the discharge takes place; (2) the effluent discharge must not result in the creation of floating foam on the surface of the receiving waters; and (3) the effluent must not show greater than 50 % mortality at 100 % concentration for any discharges entering into the receiving water. Although not stated in the regulations, our dealings with the province have indicated a preference for an operation that does not allow for the use of chemicals or chemical processes that could result in a toxic discharge episode from a process failure. For that reason, a number of the toxicity trials looked at individual process streams and specific chemical formulations, to determine the impact that could arise from a process upset.

TREATMENT PROCESSES STUDIED: The placement of the textile facility dictates the discharge area for the effluent. The effluent criteria were therefore dictated by the carrying capacity of the receiving waters. To achieve the level of treatment required for the individual installations, the studies evaluated treatment of the textile wastes by various methods: (1) direct discharge of the waste streams, or component waste streams, without any form of treatment; (2) pretreatment of the waste streams for the removal of gross solids, adjustment of pH, and flocculation and sedimentation of coloured matter; (3) discharge without pretreatment to existing municipal sewage treatment plants using either aerated lagoons or activated sludge processes; (4) combinations of pretreatment with subsequent effluent polishing at the municipal treatment works; and (5) treatment at on-site industrial waste facilities, with final treatment at municipal works.

The studies conducted at the laboratory scale used standard bench scale processes to evaluate the effectiveness of the treatment on both the individual waste streams from and industry and the combined plant effluents. The potential for toxicity from the waste streams were evaluated using three levels of toxicity screening, all of which had a specific objective: (1) standard OUR tests (Oxygen Uptake Rate) were conducted to determine if the waste had a specific concentration at which point toxicity would occur within a biological treatment plant from a sudden and irreversible discharge; (2) biological degradation studies were completed on the various mixtures at different hydraulic and solids retention times to determine if the biological treatment plants could acclimatize to the wastes, or whether the wastes would result in eventual failure of the plant from selective toxicity (ie. destruction of colonial ciliates); and (3) routine effluent toxicity test (*Daphnia magna* and Rainbow Trout, *Oncorhynchus mykiss*) were undertaken on the virgin chemicals used in the process, the effluent streams from the individual processes, the pretreated effluent streams and the final effluent after any polishing stages.

Where a specific waste stream or chemical was identified as having an unacceptable level of toxicity, the alternative chemical formulations were then examined. If no alternative was available then means to control the toxicity were evaluated. Each of the process streams were evaluated at concentrations expected from the plant, plus concentrations that could arise during an unforseen process upset.

TEXTILE PLANTS STUDIED: The three major groups of textile plants studied included: (1) an integrated printing and dyeing operation using cotton and polyester materials, a wide range of interim fabric process stages (singeing, starching, PVA, CMC, fire retardants) followed by application of a wide variety of organic dyes, several of which contained heavy metals; (2) a dyeing operation with cotton fabric only, using a limited number of processes for fabric preparation and base colour dyeing only; and (3) an indigo slashing and dyeing operation for the production of denim materials from a cotton fabric, with limited amounts of starching.

In each of the studies completed there were a number of benefits achieved from the evaluation of the toxicity of the chemicals used in the manufacturing, the processes carried on at the mill, or the operations within the treatment facilities.

At the existing mill where the studies concentrated on the development of treatment processes to combat toxicity, the tests were clearly beneficial in the following areas: (1) a number of the chemicals used in the processes were identified as being extremely toxic, providing the operators with the information necessary to approach the chemical suppliers regarding replacement chemicals or formulations; (2) process operations (ie. polyester singeing) were identified as steps that resulted in the formation of toxic compounds that were collected in the effluent after fabric washing, therefore requiring specific attention at the treatment plant (ie. powdered activated carbon); (3) pretreatment needs could be developed to handle the individual waste streams, prior to being combined in a final effluent stream, which allowed for optimization of the pretreatment, avoiding contamination of the total plant effluent; (4) the ability of the manufacturing operation and treatment plant to achieve the objectives established by the regulatory agencies could be clearly established and maintained on a routine basis; and (5) the general public could be assured of the success of the facility in meeting the "non-toxic" discharge criteria, a critical component given the dependency of the area residents on the aquatic resources.

The same general benefits were also available to the promoters of the two proposed textile facilities, allowing changes to be made early in the process, thus avoiding the cost for expensive remedial treatment. In particular, testing conducted at the plants in the United States and Hong Kong enabled the developers and the regulator agencies to work together to achieve the following: (1) individual process streams were evaluated enabling the owners to identify which processes produced a highly toxic effluent, resulting in adjustment to the processes proposed for the facility; (2) chemical formulations were checked and tested, allowing the chemical suppliers the opportunity to suggest alternative formulations; (3) treatment needs could be determined early in the process, allowing for the inclusion of these costs in the overall planning for the facility, particularly where process changes could be compared to treatment needs; (4) the regulatory agencies and general public could be assured early in the review process of the general acceptance of the facility relative to the achievement of the effluent objectives; and (5) baseline conditions could be established for future examinations of the manufacturing processes, in anticipation of future phases to the production facilities.

TOXICITY PROBLEMS IDENTIFIED: The results of the investigations were unique in that a number of areas were identified as being critical to the achievement of the "non-toxic" effluent. Conducting the tests on the individual process streams as well as the combined plant effluents allowed us to study the synergistic impact of toxic components, the toxicity reduction from total plant effluent dilution, the production of toxins within the process, and the effectiveness of various waste treatment alternatives.

A number of sources of toxicity were identified as a result of the combination of the process investigations and toxicity tests completed: (1) pH imbalances were common for individual process streams, thus enabling operations to plan for pH adjustment, equalization prior to discharge, or establishment of alarm conditions; (2) high strength organic wastes were shown to impact on the toxicity tests from oxygen depletion, demonstrating the need for adequate biological treatment prior to discharge and containment in the event of spills; (3) high ionic strength chemicals resulting in imbalances to the osmotic pressure in the effluent were identified and corrected (ie. chlorides, sulphates); (4) specific toxic chemicals (ie. chrome, chlorine) were identified and formulation changes were made or treatment processes added; (5) by-products from the processing of the fabric (ie. singeing) could be traced back to areas in the production facility; and (6) failures in the treatment plant could be characterized relative to the impact on toxicity results.

Ultimately, the chemical formulations approved for use and the treatment processes selected, were based on the results of the toxicity evaluations. In particular instances, spills response programs were developed to handle potentially toxic episodes, such as the addition of PAC (powdered activated carbon) to the effluent lagoons.

TOXICITY TESTS COMPLETED: Process testing for the mill evaluations were conducted either at the mill sites in Hong Kong or the United States, or at Pollutech's Oakville process engineering laboratory. All samples for toxicity evaluations were transported to Pollutech's Sarnia biological research facility. Standard toxicity testing protocols were used that responded to the requests of the regulatory agencies as well as those that provided insight into the potential for effluent toxicity from the manufacturing processes. For example,lethality toxicity tests that incorporated other sublethal endpoints, such as an EC_{50} or TLOE (Total Loss of Equilibrium), were of particular importance to the process group to identify areas that required further attention. By paying attention to these sublethal responses, process upsets and toxicity problems could be expediently isolated. The standard LC_{50} tests were of greater importance to the regulatory agencies as a result of the lethality compliance requirement.

The *D. magna* 48 hour acute lethality test highlighted both mortality data and mobility. The standard Rainbow Trout 96 hour lethality test provided data regarding partial and total equilibrium loss.

The tests conducted are summarized as follows: (1) *D. magna* 48 hour acute lethality testing provided data on acute as well as sublethal response (ie. loss of mobility); (2) Rainbow Trout 96 hour acute lethality testing provided data on acute as well as sublethal responses (ie. Total Loss of Equilibrium -TLOE).

PROGRAM TEST RESULTS: To demonstrate the results that were achieved from the individual test programs, we have highlighted important findings to the process investigations for the three case studies.

In the first case at the existing textile facility, Pollutech conducted treatability testing and toxicity evaluations to optimize the treatment process. In the latter stages of the project, Pollutech assisted with the design, construction and commissioning of an on-site toxicity laboratory at the textile plant. During the commissioning of the treatment plant, a series of tests provided the following results:

Date	*Daphnia magna* 48 hour EC^{50}[1]	*Daphnia magna* 48 hour LC_{50}[2]	Rainbow Trout % TLOE [3]	Rainbow Trout Pass/Fall [4]
Nov. 9	no immobility	non-lethal	0	non-lethal
Nov. 12	not run	not run	0	non-lethal
Nov. 15	no immobility	non-lethal	0	5 %
Nov. 16	no immobility	non-lethal	0	non-lethal
Nov. 23	no immobility	non-lethal	100	50 %
Nov. 23	no immobility	non-lethal	100	60 %

The effective concentration of the chemical or effluent which causes immobility in 50 % of the test organisms.

[2] The lethal concentration at which mortality occurs to 50 % of the organisms.

[3] Total Loss of Equilibrium.

[4] % Mortality.

The test results were critical in that the operating permit during the commissioning period allowed the plant exceed the BOD_5 and Suspended Solids effluent criteria but explicitly disallowed any releases of toxic effluents. The availability of the results at the site allowed staff time to divert the treated effluent to the holding lagoon where it could be further treated with activated carbon to reduce the toxicity. Activated carbon had been identified in the lab scale trials as a viable means to control toxicity form the singeing operation. As a result of this episode, the production team made changes to the process that corrected this problem.

It is important to note that the *D. magna* tests, although they provided a good screening tool in the design stage of the project, did not highlight the effluent toxicity associated with the singeing operation. The protocol for conducting toxicity tests on the treated effluent and the individual process streams became an important aspect in the operation and compliance monitoring for the facility.

In the second case study, the similarities of the existing process in northeast New Brunswick discharging to the Atlantic Ocean was evaluated against a proposed facility in northwest New Brunswick discharging to an inland river. Three sets of samples from supposedly "similar" operations were evaluated with the *D. magna* 48 hour LC_{50} protocol. The results obtained are detailed as follows:

Sample	Northwest Plant 48 hour LC_{50} (% by volume)	Northeast Plant 48 hour LC_{50} (% by volume)
A	<0.25	11.3
B	<0.25	>100
C	<0.25	34.1

For the northwest plant, no specific 48 hour LC_{50} value could be obtained as 100 % toxicity occurred at the lowest concentration tested (0.25 % by volume). Toxicity was observed to occur very rapidly. Even at the lowest concentrations studied, 60 to 100 percent of the Daphnia were immobile within 24 hours of initiating the test. These preliminary screening trials showed that the effluent from the Northwest (proposed) facility was at least 136 time more toxic than the existing facility. As the proponents of the mill had claimed that the operation was "non-toxic" there was a definite change in the operations plan for the proposed facility.

In the third case study, currently in progress, the individual chemical components of the process are being evaluated along with the process streams, the final effluent, and mixtures of the wastes with other industrial and municipal streams. Each chemical is being tested at the expected concentration in the process, as determined by a mass balance, and at three times the expected concentration. The preliminary results of the *D. magna* tests are as follows:

Chemical	Daily Usage (lbs)	Yarn Uptake (%)	Chemical (mg/L)	3 X (mg/L)	EC_{50} (mg/L)	LC_{50} (mg/L)
Indigo Dye	120	85	57	172	>200	>200
Sodium Hydrosulphite	240	85	115	344	222	222
Soda Ash	32	85	15	46	>50	>50
Subitol San	40	85	19	57	1.1	1.1
Tapioca Flour	882	85	421	1264	>1300	>1300
Bev 4118	100	85	48	143	>150	>150

The test results clearly show that one particular compound (Subitol San) will create a toxicity problem if the formulation can not be modified or the waste can not be appropriately treated.

Ten treatment processes and combinations were evaluated with both the *D. magna* and the Rainbow trout test protocols. The tests were undertaken to determine the efficiency of the waste treatment process with the various alternatives under the following conditions:

(1) the treatment plant receiving domestic sanitary sewage only, representing the base case if no industries discharged to the facility; (2) the treatment plant receiving sanitary waste plus an existing effluent stream from a local pulping operation; (3) the treatment plant receiving sanitary sewage plus the proposed textile effluent, without the pulping waste; and (4) the combination of sewage, pulping waste and textile effluent.

Each test was completed at 50 %, 100 % and 200% of the expected feed concentration, with a 12 and 24 hour HRT (hydraulic retention time) and a 10 day, 20 day and 30 day SRT (Solids Retention Time). These conditions had been previously identified as the critical design parameters for the biological treatment facility.

Preliminary test results are available on the effluents collected during the acclimatization period of the biological treatability tests, as depicted below:

Bioassay Test	**Rainbow Trout 96 hour Pass/Fail**	***Daphnia magna*** **48 hour LC_{50} (% by volume)**
Sewage Only	pass	84
50 % Textile	pass	75
100% Textile	pass	80
200 % Textile	fail[1]	>100
50 % Pulp Waste	pass	85
100% Pulp Waste	pass	NT
200 % Pulp Waste	pass	NT
50 % Mixture	pass	>100
100% Mixture	pass	NT
200% Mixture	pass	NT

[1] 60 % mortality at 100 % effluent concentration

The effluent toxicity results showed that there was a toxicity problem associated with the sanitary sewage alone when it had not been fully treated. The textile and pulp mill wastes showed signs of toxicity that can be addressed with adequate biological treatment, provided the concentrations are not excessive. The individual chemical toxicity results assisted in the identification of the Subitol San as a major toxicity concern.

The test results that we have obtained from the case studies have demonstrated that the textile manufacturing process would have to be operated to ensure that there was: (1) no dumping of acids or bases; (2) no production with sulphide based dyes; (3) treatment after singeing of polyester fibres; (4) control of dyes with trace metals; (5) no spillage of high strength organics, and (6) no treatment plant failures.

CONCLUSIONS

The studies conducted to date have been critical to the evaluation of the environmental impact of these textile facilities. In general the tests have shown that: (1) the *D. magna* is a useful screening tool for identifying potential problems with individual chemicals or treatment alternatives; (2) Rainbow trout testing is required for compliance testing as not all effluents responded equally to *D. magna* and Rainbow Trout toxicity tests; (3) the investigator must screen component streams to ensure that the treatment facilities are not designed to treat large volumes of waste that are created toxic by a small side stream; (4) it is essential to account for process upsets in the studies resulting in the formation of toxic streams from otherwise normal operations; (5) the study must evaluate both raw and treated wastes to provide information on the toxicity reduction with treatment and the potential impact form treatment plant failures; and (6) the evaluation must screen a series of concentrations for process chemicals, given the wide range of process changes experienced at the operating facilities.

THE ACUTE LETHAL TOXICITY OF INDUSTRIAL EFFLUENTS COLLECTED UNDER ONTARIO'S MISA PROGRAM TO RAINBOW TROUT AND TO *Daphnia magna*. J.T. Lee, G.F. Westlake, S.G. Abernethy, D. G. Poirier and M.C. Mueller. Ontario Ministry of Environment and Energy, Etobicoke, ON, M9P 3V6.

Under the Ministry's Municipal-Industrial Strategy for Abatement, major industries including Electric Power Generation, Industrial Minerals, Inorganic Chemical, Iron and Steel, Metal Casting, Metal Mining, Organic Chemical, Petroleum Refining and Pulp and Paper sectors, were required to conduct acute lethality toxicity tests using rainbow trout and *Daphnia magna* on samples of their effluents. The regulation also required chemical characterization of the same effluent discharges. When the monitoring was completed, over 3500 samples were collected and tested to both trout and *D. magna* from 208 effluent discharge sites. Over 50 percent of the sites had samples which were acutely lethal to trout and/or to *D. magna*. Approximately 20 percent of the samples tested were lethal to trout and 24 percent were lethal to *D. magna*. This self-monitored data produced by 14 contract laboratories representing industry had less than 1 percent invalid results. Also Ministry audit results were in agreement with this data. Comparisons with chemical characterization data suggest that pH extremes, ammonia, suspended solids, phenol, resin acids, BOD, cyanide, As, Cr, Zn, Cu, Ni were the major causes of acute toxicity.

A REVIEW OF THE TOXICITY OF METAL MINING EFFLUENTS IN CANADA. W.R. Parker[1] and S. Andrews[2]. [1] Environmental Protection Branch, Environment Canada, Atlantic Region, Halifax, NS, B2Y 2N6; [2] University College of Cape Breton, Sydney, NS.

The Metal Mining Liquid Effluent Regulations and Guidelines are intended to limit the discharge of deleterious substances from base metal, uranium and iron ore mines. The regulations apply to new, expanded, or reopened mines and the guidelines apply to mines that existed before 1977. A survey was conducted to assess the present status of the aquatic toxicity of base metal mining effluents in Canada. A second objective of the study was to determine whether revisions to the MMLER&G should include a requirement for toxicity testing and/or requirement that mining effluents are non-toxic using a standard aquatic toxicity test. At present, the Regulations do not require an effluent to be non toxic to aquatic organisms and an effluent can be within the MMLER&G chemical limits and still be acutely lethal.

Data was collected though cooperative arrangements between federal and provincial agencies and the mining industry. The data was gathered and evaluated to ascertain the effectiveness of limiting specific deleterious substances and reducing or eliminating acute lethality of effluent to aquatic organisms. The results of 1039 acute lethal toxicity tests on effluents from 72 active Canadian metal mines were collected and evaluated. Of the 1039 toxicity test results received, 599 test results met the screening criteria established for the project and underwent further evaluation. These test results consisted of 409 rainbow trout acute lethality test results, 148 *D. magna* test results and 42 Microtox^R test results.

For the rainbow trout tests, 336 (82%) tests were non-acutely lethal and 357 (87%) of the samples were in compliance with the grab sample limits of the Metal Mining Liquid Effluent Regulations. There was not direct correlation between toxicity and compliance. Of the 357 samples that were in compliance, 56 failed the toxicity test (less than 50 % survival in full strength effluent). Of the 52 samples that were not in compliance with the regulatory grab sample limits, 35 passed the toxicity test.

A similar pattern was observed for the *D. magna* tests. Of the 148 samples results reported, 134 (91 %) were in compliance and 126 (85 %) were non-acutely lethal to the *D. magna*. Of those samples in compliance, 19 failed the toxicity test while of the 14 samples that had contaminant levels in excess of the regulatory grab sample limit, 11 passed the toxicity test. The number of mine effluent samples tested using Microtox^R and reported during this study was 42. Of these, 36 were in compliance with the regulatory limits and 40 were non-toxic as measured by the Microtox^R system.

The survey indicated that the majority of base metal mines in Canada were discharging effluents that were not acutely lethal when tested using common laboratory toxicity test methods. The number of samples that were non-acutely lethal was similar to the number of samples that were in compliance with the chemical limits specified by the Metal Mining Liquid Effluent Regulations. Proposed revisions to the federal mining regulations should consider incorporating the requirement that mining effluents not be acutely lethal using standard laboratory toxicity tests.

THE SUBLETHAL EFFECTS OF PETROLEUM REFINERY EFFLUENTS: INTER AND INTRA REFINERY VARIATIONS. J.P. Sherry, B.F. Scott, E. Nagy and B.J. Dutka. Environment Canada, National Water Research Institute, Burlington, ON, L7R 4A6.

A battery of toxicity tests was used to assess the sublethal potency of effluent from an

Ontario refinery (A) over a six week period; the potencies of effluents from two other refineries (B and C) were also assessed. In a preliminary study we had detected sublethal toxicants in effluent samples from refineries B and C. It was unclear, however, whether the positive samples were typical of those refineries or of refineries in general. In this follow-up study whole effluent samples from refinery A were tested five times, at approximately 14-day intervals, for sublethal toxicity. A battery of tests that included bacterial, algal, plant, cladoceran, a sub-mitochondrial particle based assay, and fish based tests was used. The genotoxic potency of the samples was also assessed. Eggs and newly hatched larvae of rainbow trout were exposed to Refinery A effluent for 25 and 21 days respectively. The battery of tests was also used to assess two effluent samples from each of the previously studied refineries (B and C). Several chemical parameters including ammonia-nitrogen, volatile components, heavy metals, PAHs, and alkanes were measured in the effluents. Only the Microtox test detected acute toxicity in the effluent samples. The development of the fish eggs was not affected by long term exposure to refinery A effluent, at least until the embryo eyes had differentiated, at which stage the experiment was aborted. Neither did the 21 day exposure of newly hatched alevins detect the presence of toxicants. The fathead minnow test, a chronic exposure test, detected effluent induced mortality in two out of five samples from refinery A. Both of the refinery B samples affected the growth of fathead minnows; one of those samples was positive in the seed germination test. The refinery C samples were marginally toxic to *Selenastrum capricornutum*. All effluent samples affected the reverse electron transport system in the sub-mitochondrial particle assay at a 10 X concentration - the 1 X samples were negative. We shall discuss our results in relation to current regulations, the past performance of Ontario refineries, and the need for ecologically meaningful indicators of effluent toxicity.

EXTRACTABLE ORGANOCHLORINES (EOCls) IN FISH EXPOSED TO BLEACHED KRAFT MILL EFFLUENT. H.E. Sonnenberg[1], J.H. Carey[2], P.V. Hodson[2] and K.R. Solomon[1]. [1]University of Guelph, Department of Environmental Biology, Guelph, ON, N1G 2W1; [2]Environment Canada, Canada Centre for Inland Waters, Burlington, ON, L7R 4A6.

Several studies have found extractable organochlorines (EOCl) in fish from pulp mill effluent receiving waters. In recent years, new pulp bleaching technologies that reduce the production of organochlorines in pulp mills have been introduced. The impacts of this new technology on the EOCl concentrations in fish are not well known. We analysed EOCl in wild white sucker (*Catostomus commersoni)* from two pulp mill locations (Jackfish Bay and St. Maurice River) and one "pristine" location (Gatineau River). The amount of EOCl in the fish was determined by neutron activation analysis. Archived fish from the St. Maurice River caught prior to the bleaching process changes were also analyzed. Results indicate that EOCl levels in effluent exposed fish (16 *ug*/g) are significantly higher than in unexposed fish (0.5-1.5 *ug*/g). EOCl levels in exposed fish from the bay site were much more variable than those in fish from the exposed river location. Surprisingly, there was little difference in EOCl levels between fish from before and after the bleaching process changes. It has been suggested that much of the EOCl in fish exposed to pulp mill effluent is composed of chlorinated lipid. To evaluate this suggestion, EOCl in lipid and non-lipid fractions in fish from both exposed and non-exposed locations was determined. Hydrolysed lipid fractions were also examined for the presence of chlorinated fatty acids.

TOXICITY TEST METHOD DEVELOPMENT
SESSION CHAIR: P. RIEBEL

MEASURING CHRONIC TOXICITY USING LUMINESCENT BACTERIA. A.A. Bulich and H. Huynh. Microbics Corporation, 2232 Rutherford Rd., Carlsbad, CA, 92008.

Bioassays using luminescent bacteria are routinely used to assess the acute toxicity of environmental samples. Two physiological characteristics of these test organisms, a short division cycle (120 minutes) and the inducible luciferase pathway, provide useful attributes for measuring **chronic toxicity**. Freeze-dried luminescent bacteria inoculated into appropriate growth medium, initiate reproduction while concurrently inducing a complex series of metabolic pathways resulting in the production of the bioluminescence. Toxic chemicals which inhibit any aspect of the reproductive cycle or induction of light production are detected in low concentrations. This new test method is based upon a detailed understanding of growth requirements and biochemistry of this organism and the mechanism of luciferase induction. Consequently, a defined test medium was developed which supports cell growth and luciferase induction yet does not mask toxic substances. Test organisms are inoculated into cuvettes containing 1 ml each of growth medium containing dilutions of test sample. After 18 hrs incubation at 27°C, control cuvettes show relatively high light levels while toxic sample dilutions exhibit decreasing light levels. Details of the test protocol are presented along with an assessment of test precision. Sensitivity data from this chronic toxicity test are presented and compared with the Microtox® acute test and *Ceriodaphnia dubia* chronic toxicity test. In general, this test method is about 20 times more sensitive than Microtox and exhibits sensitivity similar to *C. dubia* for the samples tested.

BIOAVAILABILITY OF CONTAMINANTS FROM CHEMICALLY-FRACTIONED HAMILTON HARBOUR SEDIMENT TO A SUITE OF AQUATIC ORGANISMS. L.H. McCarthy[1,2], C.I. Mayfield[1,2] and R.L. Thomas[2]. [1]Department of Biology, and [2]Waterloo Centre for Groundwater Research, University of Waterloo, Waterloo, ON, N2L 3G1.

Although a "whole" sediment may be toxic to a variety of organisms, the chemical group or fraction causing the toxicity is often unknown. In the past, researchers have implicated those chemical(s) present in the highest concentration as the culprits responsible for the noted adverse impact. However, rarely has conclusive proof been shown that these contaminants are indeed the causative agent(s) of toxicity. The toxicity of bulk sediment from Randle Reef in Hamilton Harbour was assessed using a suite of aquatic organisms, which included the phytoplankton *Selenastrum capricornutum*, the zooplankter *Daphnia magna*, the amphipod *Hyalella azteca*, and the fathead minnow *Pimephales promelas*. Contaminants in the sediment included heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). When the bulk sediment proved toxic to these organisms, a bioassay-driven frationation protocol was implemented to identify the toxic chemical agents in the sediment. The fractionation methodology utilized dichloromethane in Soxhlet extractors and silica-gel chromatography for separation. Extraction and separation of the Hamilton Harbour sediment narrowed a causative agent of toxicity to a nonpolar organic fraction and chemical analysis identified PAHs as the dominant compounds. The study concluded that bulk chemical characterization is inadequate in predicting potential adverse impact of contaminated sediment, and that fractionation schemes should be implemented in future assessments of the toxicity of sediment.

A *Daphnia magna* AND *D. pulex* IN-SITU ASSESSMENT TO DETERMINE THE EFFECTS OF INDUSTRIAL, MUNICIPAL AND RUN-OFF DISCHARGES INTO THE ST. CLAIR RIVER ON SURVIVAL AND REPRODUCTION. T. Moran[1], D. Adams[1] and C. Beckwith[2]. [1]Pollutech Environmental Limited, 1149 Vanier Road, #4, Sarnia, ON, N7S 3Y6; [2]Imperial Oil, Products and Chemicals Division, P.O. Box 3033, Sarnia, ON, N7T 7Z4.

ABSTRACT

The International Joint Commission has indicated the St. Clair River as one of the "Areas of Concern" within the Great Lakes. To determine the environmental impact of ongoing discharges to the St. Clair River, an in-situ flow-through bioassay approach was instituted. In-situ monitoring included the use of *Daphnia magna* and *D. pulex* life-cycle testing for determining survival and reproductive rates. The experimental design included a control station located upstream of all industrial and urban inputs and a similar downstream station. A series of toxicity tests were conducted using both a flow-through and static replacement apparatus that allowed for monitoring of the river water quality from routine discharges and spill events.

INTRODUCTION

In a healthy ecosystem, organisms should be capable of growth, reproduction, and survival. Reproduction or fecundity has been indicated as one of the most sensitive indicators of toxicity (Sutter et al. 1987). If an ecosystem has been altered to create undesirable conditions, growth or reproduction or survival can be affected. Such endpoints are estimated routinely by the use of various types of bioassays under laboratory and/or *in-situ* conditions. The design of the biological monitoring program discussed in this paper incorporates the endpoints of growth, reproduction and survival by utilizing the two daphnid species *D. magna* and *D. pulex*.

This paper focuses on the data accumulated from the *Daphnia* study conducted during the summer of 1992 as part of the Lambton Industrial Society's (L.I.S.) ongoing biological monitoring program. Specifically the objectives of this program were: to determine whether differences existed between stations as defined by the biological response of the test organisms (ie. differences in reproduction); to determine whether there was a difference between raw water and filtered water (ie. daphnids in filtered water reproduce better than in raw water); to determine whether differences existed between species.

MATERIALS AND METHODS

The experimental design followed a protocol for a 21-day test of *D. magna* survival and reproduction (ASTM 1990). The method used was adapted from the protocol to include two species of daphnids with the testing being conducted in a flow-through apparatus as well as a daily static replacement apparatus.

Experimental Design: The 1992 study was a 2^4 experimental design with location (upstream/downstream), species (*D. magna/D. pulex*), flow (flow through/static replacement), and filtration (filtered/raw) as the factors. Two species of water flea, *D. magna* and *D. pulex* were contained within flow-through and static replacement systems located upstream and downstream of the Sarnia/Lambton study area (Fig. 1). At each location the daphnids were exposed to both filtered and raw water. Data from the two biomonitoring stations were compared to determine the net effect of river water quality on daphnid survival, growth and reproduction.

Test Organisms: The two test species, *D. magna* and *D. pulex* were obtained from Pollutech's main cultures in the lab. Each species was acclimated to St. Clair River water for 4 months (≥ four generations) and fed a diet of two algal species, *Selenastrum capricornutum* and *Chlorella fusca* according to the ASTM (1990) methods. Each test organism was obtained from the third brood of the acclimated adult *Daphnia* as suggested by Environment Canada (1990b). Prior to the initiation of the test, young daphnids (neonates) obtained from the various culture jars were pooled and split into two vessels; one for upstream and one for downstream. Three-day old neonates were used to begin the 21-day test in run #1. For run #2 5-day old *D. magna* and 4-day old *D. pulex* were used to begin the experiment. Previous studies (Pollutech, 1989, 1991 and 1992) determined that neonates should be 3 to 5-days old before starting the in-situ experiments. One neonate was placed into each test vessel. Daphnids were transferred using a modified glass pipette.

Apparatus: The apparatus for this project was originally developed by Pollutech during 1990 for the L.I.S. biomonitoring program. River water for the experiment was supplied by centrifugal pumps. The water was distributed throughout the stations using PVC and CPVC pipe.

To obtain filtered water, river water was passed through a series of coalescing filters in the order 80 µm, 30 µm and 5 µm. The filter housing allowed easy viewing of the filter cartridges to determine whether they needed cleaning or replacement before flow was significantly reduced. The filter media complied with United States Food and Drug Association regulations as indicated by the manufacturer, Balston. At each station water entered into two separate header tanks through float valves, one for filtered water and the other for raw water. Each header tank was equipped with a float valve. Each header tank was also equipped with separate valves to supply and control flow into two individual aquaria. Water entered the aquaria through a CPVC diffuser pipe positioned against the back wall of the tank. In so doing, disturbance to the daphnids was minimized and even water flow throughout the aquarium was achieved. Each 40 L glass aquarium was equipped with a standpipe draining water from the top of the tank so that a constant water level was maintained.

In the middle of each aquarium a plexiglass divider was installed and sealed using "aquarium safe" silicone. Into each divider 10 daphnid chambers were placed. Each chamber held one daphnid throughout the test period. The chambers were modified test tubes. The closed end of the tube was cut away and covered with 200 µm Wildco Nitex mesh. The open end was closed by a rubber stopper fitted with a CPVC tube through its centre. The rubber stoppers for each aquaria were labelled 1 through 10 in order to identify the appropriate replicate and allow randomization. Nitex mesh was also used to close the intake end of the CPVC tube. By covering both ends of the test tube with Nitex mesh, water was able to pass through the tube exposing the contained *Daphnia* to a continuous water supply. The rubber stoppers also served to seal the holes in the dividers forcing water to pass through each chamber. Chambers were easily removed from the divider for observations and cleaning.

The static replacement trials were conducted parallel to the flow-through trials at each station. The static replacement tests were conducted in similar chambers (50 ml test tubes) as the flow-through experiments. Water samples were collected from 24-hour composites from the same source as in the flow-through tests. Replacement of solutions was conducted daily. The test chambers were maintained in a flow-through river water bath that maintained the same test temperature as the flow-through test apparatus.

Because of the experimental design, foraging for algae was impossible, therefore a diet of algae was supplemented. The flow-through tanks were fed every second day by placing 1.5 litres of algae (50% *S. capricornutum* and 50% *C. fusca*) into each header tank. The static replacement system was fed 9-12 ml of the algae mixture daily (depending on cell density) per 500 ml of water. Feeding rates were based on ASTM (1990). The two test periods for the 21-day survival and reproductive study started July 10, 1992 (run #1) and August 4, 1992 (run #2).

Statistical Methods: Analysis of Variance (ANOVA) was used to detect differences in reproduction in response to different treatments. The total number of neonates, by replicate, produced in each group were analyzed to detect differences in the reproductive capacity. Mortality was taken into account in the analysis; adult mortality occurring prior to the end of the test period reduced the number of neonates in a test chamber and subsequently in that test treatment. Reproductive data (the total number of neonates produced) were analyzed for statistical significance.

The data, which is count data, is not well suited to analysis under normal assumptions. However, barring the use of constructed variables or a non-parametric analysis no other approach is suitable. The use of constructed variables was avoided due to problems with interpretation of hypotheses. Non-parametric analyses for a complicated factorial are difficult as well as lacking in power. The lack of normality in this case did not prove to be a great hindrance since the primary purpose of the experiment was hypothesis testing as opposed to the generation of a predictive model.

In general, residuals exhibited more kurtotic than skewed behaviour, thus a cell-means based approach was justified. The model building method was that of reduction. Once the final model was determined, transformations were applied to the model preceding the final model to ensure that the transformation had no effect on the significance of the variables. The final model fitted used the appropriate transformation. The final model is as follows:

log (# neonates) = Species + Flow + Location + Filtration + Interactions
where Species =*D. magna* versus *D. pulex*; Flow =Flow-through versus Static Replacement; Location =Upstream Station versus Downstream Station; Filtration =Raw Water versus Filtered Water.

RESULTS AND DISCUSSION

Data Presentation and General Trends: The endpoints measured include adult daphnid survival and number of offspring (neonates) produced per breeding adult. These endpoints represent both acute (short-term) and chronic (longterm) responses to water quality. Though the survival results were not statistically analyzed a summary is provided in Table 1 for each run. Detailed tabulated results can be found in Pollutech (1993).

Table 1 - Summary of Daphnid Survival Results Run #1 and #2

RUN #	SPECIES	% SURVIVAL							
		UPSTREAM STATION				DOWNSTREAM STATION			
		Flow-through		Static Replacement		Flow-Through		Static Replacement	
		Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
1	*Daphnia magna*	50	80	100	100	90	70	100	100
	Daphnia pulex	80	80	100	80	80	80	90	60
2	*Daphnia magna*	10	60	50	50	80	50	20	80
	Daphnia pulex	40	60	100	80	60	20	100	100

Table 2 - Total Number of Neonates Run #1 and #2

RUN #	SPECIES	TOTAL NUMBER OF NEONATES							
		UPSTREAM STATION				DOWNSTREAM STATION			
		Flow-through		Static Replacement		Flow-Through		Static Replacement	
		Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
1	*Daphnia magna*	408	808	578	568	688	570	646	602
	Daphnia pulex	142	92	1042	777	323	190	1093	784
2	*Daphnia magna*	6	317	220	176	332	150	70	267
	Daphnia pulex	45	76	895	641	306	83	833	847

Overall subjective comparisons would indicate that survival was better in run #1 than in run #2. Subjective comparisons would also suggest the static replacement system to have similar survival upstream and downstream; whereas the flow-through system illustrated comparable results at both stations but with slightly better survival downstream.

Table 2 summarizes the total number of neonates produced for run #1 and #2. Neonate reproduction for replicate is utilized in the statistical analysis.

Table 3 - Anova Summary of Results Run #1 and Run #2

SOURCE	Summary of ANOVA Results	
	Run #1 (p)	Run #2 (p)
Flow	SIG (p=0.0001)	SIG (p=0.0001)
Filtration	SIG (p=0.0438)	NS (p=0.4236)
Species	NS (p=0.1044)	SIG (p=0.0001)
Location	NS (p=0.0664)	SIG (p=0.0216)
Flow*Filtration	SIG (p=0.0059)	NS (p=0.9850)
Flow*Species	SIG (p=0.0001)	SIG (p=0.0001)
Flow*Location	NS (p=0.5369)	NS (p=0.1307)
Filtration*Species	SIG (p=0.0002)	SIG (p=0.0007)
Filtration*Location	SIG (p=0.0100)	NS (p=0.2252)
Species*Location	NS (p=0.4593)	NS (p=0.1702)
Flow*Filtration*Location	SIG (p=0.0440)	SIG (p=0.0001)

SIG = Significant NS = Not Significant

Run #1 - Statistical Analysis: The general linear model used resulted in a non-significant 4-way interaction. Consequently the model was reduced to a 3-way interactions model. The 3-way interaction is shown to be only marginally significant (p=0.04) for "flow*filtration*location". In view of the marginal significance of the 3-way interaction it is likely that the interaction is dominated by the very significant effect of flow (flow-through versus static replacement). The model is rerun with only 2 way interactions included. The significance of the variables remains constant when the 3-way interaction is removed from the model (Table 3).

The significant 2-way interactions shown in Table 3 appear to be dominated by the significant effects of flow and filtration. The results do indicate more neonates produced in the static replacement system. More neonates are produced when filtered water is used in a flow-through setting but more are produced when raw water is used under a static replacement system. *D. magna* produce more neonates in the flow-through system while *D. pulex* produced more neonates in the static replacement system.
Although a significant "filtration*location" interaction was found it is likely this significance is due to the significance of the filtration effect as opposed to any station effects. *D. magna* produced more neonates when the water was filtered but *D. pulex* produced more neonates in raw water. Statistical Analysis Run #2: The general linear model used resulted in a non-significant 4-way interaction. Consequently the model was reduced to a 3-way interactions model. The 3-way interaction is shown to be highly significant (p=0.0001) for "flow * filtration * location". In run #1 this interaction was only marginally

significant (Table 3).

An obvious effect of the exposure method (static replacement versus flow-through) occurs with both *Daphnia* species with the static replacement system producing more neonates. Daphnia at the downstream station produced more neonates. These conclusions verify the significant main effects. Construction of 2-way tables for flow (exposure system) versus location for each level of filtration do not show any interaction supporting the main effect conclusions.

Construction of 2-way tables for exposure system versus filtration method for each station highlights the interaction. More neonates are produced in the raw water at the downstream station while at the upstream station more neonates are produced when the water is filtered and subsequently fed. These observations verify the hypothesis that more food is available in the raw water at the downstream station. When this water is filtered the difference between upstream and downstream stations is negligible. Thus the increased neonate production at the downstream station is likely the consequence of increased food availability.

Flow (Static Replacement versus Flow-through): In each run several significant flow interactions were found with filtration and species. Of the main effects flow was highly significant in both runs. When comparing the static replacement system to the flow-through system in runs #1 and #2, it was discovered that reproduction was more successful in the static replacement system. One reason for this occurrence may be the fact that the flow-through systems required more handling. Because of the continuous supply of water going through the flow-through system, the test tubes were prone to increased build-up of algae. As a result the test tubes had to be cleaned more often in order to see the daphnids, and the mesh covering the ends of the tubes had to be scrubbed every second day to allow water to flow through continually. The tubes also had to be removed from the tanks and placed in water-filled beakers in order to count the neonates. A second reason for less successful reproduction in the flow-through systems may be that the daphnids had to expend more energy during locomotion in the flow-through current, which may have left less energy for neonate production. On the other hand, the daphnids in the static replacement system had no water current to contend with and the system was fairly easy to maintain. No algae accumulated in the tubes and mesh was not used, therefore no cleaning or scrubbing was needed. Disturbance of the tubes was also minimal when neonates were counted.

Filtration (Raw Water vs. Filtered Water): Two-way interaction comparisons illustrated that reproduction in the raw water treatment was better downstream. When the water was filtered the difference between upstream and downstream stations was negligible. This tends to verify that more food is available in the raw water at the downstream station. These results would also suggest that the available food at the downstream station had no detrimental effect on reproduction.

Reproduction in run #1 was significantly better (but only marginally) in the raw water when compared to the filtered water. In run #2 reproduction was slightly better in the raw water, but there was no significant difference statistically. This increased reproduction is likely due to increased food availability (Cox et al. 1992). The filtered water treatment would have allowed less natural food to enter the test chambers making the daphnids rely on the supplemental food as their main diet source. On the other hand the daphnids in the raw water would have had more food available to them, as well as having a greater diversity of food types. The hypothesis that filtered water could have led to improved neonate production because of the filtering out of suspended solids, does not hold true in this case.

Species (*D. magna* vs. *D. pulex*): Two-way interactions show that *D. pulex* reproduction in the flow-through system is poor. For example in run #1, *D. magna* produced a mean number of 61.9 neonates in the flow-through and 59.9 in the static system. Similarly *D. pulex* produced a mean of 92.4 in the static system, but in the flow-through system *D. pulex* only produced a mean of 18.7 neonates. These results demonstrate that *D. pulex* may be more sensitive to handling than *D. magna*.

Results show that reproduction was marginally better, although still statistically significant, with *D. magna* in run #1. In run #2, neonate production was significantly greater with *D. pulex*. The main reason for this may be due to the fact that *D. magna* used in the second run did not appear as healthy as in the first run. About two days after initiating the test, all *D. magna* at both stations looked smaller than normal and they also appeared to be "more white" than usual. This change in health of the test individuals in run #2 may be the result of the breeding culture(s) in the lab, and most likely did not reflect the quality of the St. Clair River[2].

Location (Upstream Station vs. Downstream Station): Statistical analysis of two-way interactions revealed that more neonates were produced in the raw water system downstream, while at the upstream station, more neonates were produced when the water was filtered. General trends also show that adult survival was also somewhat better downstream. In run #1 there was no statistical difference between upstream and downstream reproduction. In run #2, there was a significant difference in reproduction with more neonates being produced downstream. This tends to indicate that the downstream water did not have a detrimental effect on the daphnids when comparing reproduction between the upstream and downstream stations. In fact the downstream water seemed to enhance overall reproduction.

These observations lead to the hypothesis that more food is available in the raw water at the downstream station. When the water is filtered, the difference that exists between the upstream and downstream stations is negligible. Thus the increased neonate production at the downstream station is likely the consequence of increased food availability. Food availability may be increased downstream because of nutrient enrichment of the water from sources such as runoff, municipal treatment effluent, septic tank/weeping bed seepage, as well as tributary discharges.

Station effects may also be confounded with temperature as 1-2°C higher temperature periodically occurred at the downstream station compared to the upstream station. Temperature has a Q_{10} of ≈ 2 for invertebrates which could result in a 5% increase in reproduction over the 21 day test period. Differences in water temperature between locations may be due to runoff, insolation (solar energy), or discharges of warm water from local industries. During the trials water temperatures ranged between 18 and 22°C.

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[2] opinion based on personal experience

CONCLUSIONS

The following conclusions made based on the results of this study were more neonates were produced in the raw water system downstream, while at the upstream station, more neonates were produced when the water was filtered. This conclusion based on reproduction lead to the hypothesis that more food is available in the raw water at the downstream station. When the water is filtered, the difference that exists between the upstream and downstream stations is negligible. Thus the increased neonate production at the downstream station is likely the consequence of increased food availability. (3) When looking at two-way reproduction interactions between species and locations, statistically the differences were not considered significant. Adult survival downstream appears to be comparable with upstream survival based on general trends. The results do indicate the lack of any detectable detrimental effect downstream when compared to the upstream station. If these results are an indication of biotic health it can be suggested that ongoing discharges within the study area presently have no detrimental short-term or longterm effect on Daphnids.

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REFERENCES

ASTM. 1990. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. 1987. *In*: Annual Book of ASTM Standards; Water and Environmental Technology, Vol. 11.04; E 1193.

Buikema, A.L., Jr., D.R. Lee and John Cairns, Jr. 1976. A screening bioassay using *Daphnia pulex* for refinery wastes discharged into freshwater. J. Test. Eval. 4: 119-125.

Cox, E.J., C. Naylor, M.C. Bradley and P. Calow. 1992. Effect of differing maternal ration on adult fecundity and offspring size in laboratory cultures of *Daphnia magna* Straus for ecotoxicological testing. Aquat. Toxicol. 24: 63-74.

Environment Canada. 1990a. Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*. Conservation and Protection, Ottawa. Reference Method EPS 1/RM/14.

Environment Canada. 1990b. Biological Test Method: Acute Lethality Test Using *Daphnia spp*. Conservation and Protection, Ottawa. Report EPS /RM/11.

Pollutech. 1989. Lambton Industrial Society Biological Water Quality Program - 1989; Evaluation of *Daphnia magna* and *Daphnia pulex* as Useful Biological Monitors of St. Clair River Water Quality.

Pollutech. 1991. Technical Appendix to Lambton Industrial Society Biological Water

Quality Program - 1990; St. Clair River Flow-Through Studies; Rainbow Trout Egg Hatching Trials, Daphnia Survival and Reproductive Studies.

Pollutech. 1992. Lambton Industrial Society Biological Water Program - 1991; St. Clair River Flow-Through Studies; *Daphnia magna/Daphnia pulex* Survival and Reproductive Study.

Pollutech. 1993. *Daphnia magna/Daphnia pulex* Survival and Reproductive Study - 1992. Final Report Ocotber 14, 1993.

Sutter, G.W. II, R.A.E. Rosen, E. Linder and D.F. Parkhurst. 1987. Endpoints for response of fish to chronic toxic exposures. Environ. Toxicol. Chem. 6: 793-809.

SPIKING SEDIMENT WITH ORGANOCHLORINES FOR TOXICITY TESTING. M. H. Murdoch[1], D.M. Norman[2], V.M. Quintno[3] and P.M. Chapman[1]. [1]EVS Consultants, 195 Pemberton Ave., North Vancouver, BC, V7P 2R4; [2]EVS Consultants, 200 W. Mercer St., Suite 403, Seattle, WA, 98119; [3]Department of Biology, Universidade de Aveiro, Aveiro, Portugal.

Sediment toxicity testing integrates responses to sediment variables and hence does not directly indicate cause-and-effect. One tool for determining cause-and-effect is sediment spiking in which relatively uncontaminated sediment is amended with known amounts of contaminants, then tested for toxicity. Based on the concentration-response relationship(s), the relative toxicity of the spiked contaminants and their significance in sediment mixtures can be assessed. However, sediment spiking methods vary considerably. The present study details an appropriate methodology for amending sediments with a range of organic contaminant concentrations including different solvent schemes and an equilibration period. This methodology is described as appropriate because predicted and actual concentrations were similar, and responses in an acute 10-d amphipod test matched predictions and other data.

NEW TOOLS FOR ASSESSING TOXIC BIO-ACCUMULATIVE SUBSTANCES. D. Birkholz[1], G. Balch[2], D. Lintott[2] and J.S. Goudey[2]. [1]Enviro-Test Laboratories Ltd., 9936-67th, Edmonton, AB, T6E 0P5; [2]HydroQual Laboratories Ltd., #3-6125 12th St. S.E., Calgary, AB, T2H 2K1.

Aquatic organisms bio-accumulate substances directly from the water column and indirectly through the consumption of food. Persistent and hydrophobic materials generally tend to bio-concentrate (net increase) and, hence, they are more of concern from an ecological and human health risk perspective. It is often impossible to characterize and identify compounds that bio-concentrate from a routine chemical analyses of complex environmental samples. The Semi-impermeable Membrane Devices (SPMD's, Environmental Sampling Technologies) is a new tool for collecting and assessing the potential bio-availability of toxic substances that can bioconcentrate. Triolein lipid material contained within a semi-impermeable membrane sac is used as an adsorptive medium for hydrophobic materials. The device is exposed to the sample for a period of time and the lipid extracted for testing. We applied this technology to assess the bio-accumulable potential for acute and genotoxic materials in difference environmental samples. These results and other applications and limitations of this technology are discussed.

DEVELOPMENT OF AQUATIC PLANT BIOASSAYS FOR RAPID SCREENING AND INTERPRETIVE RISK ASSESSMENTS OF METAL MINING LIQUID WASTE WATERS.

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The metal mining industry faces extraordinary challenges to become environmentally sustainable. The use of non-photosynthetic organisms alone to describe environmental impact has been recognized by regulatory agencies, industry and academia as being totally inadequate both in Europe and North American. Lack of adequate testing methods for photosynthetic aquatic organisms has been recognized as a major impediment to the successful regulation and safe use of pesticides. The lack of adequate testing methods are of even more concern to the metal mining industry due to non-biodegradable nature of its waste streams. We are developing new and assessing old phytotoxicity testing methods. We expect this result in: (1) rapid and sensitive photosynthetic screening tools for the assessment of environmental hazard of on-site effluents both in freshwater in marine environments; (2) environmental relevance by including phytoplankton, submergent and emergent macrophytes, and marine macrobenthic algae; (3) risk assessment improvements for different categories of wastes and toxicity modifications caused by various environmental factors.

PRELIMINARY RESULTS OF TEST DEVELOPMENT FOR SEDIMENT TOXICITY USING MULTIPLE SPECIES OF ATLANTIC, PACIFIC AND ARCTIC POLYCHAETES.

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As part of the Canadian Environmental Protection Act, there is an Ocean Disposal Control Action Plan which calls for the development of better regulatory tools for the assessment of substances for disposal at sea. This work describes preliminary results from the development of a sediment toxicity test using multiple species of polychaetes found in Canadian coastal waters. The project commenced with a review of the literature on the use of polychaetes as test organisms, and the selection of species found in Canadian waters which might be useful as test species. The species were evaluated based upon relative fulfilment of a list of criteria. The list of criteria were: (1) sensitivity; (2) deposit feeding; (3) tolerance of fine sediment; (4) Pacific, Atlantic and Arctic counterparts; (5) tolerance of environmental change; (6) ease of collection; (7) suitable size range; (8) habitat easy to find; (9) suitable life-cycle; (10) habitat close to E C laboratories; (11) insensitive to parasites; (12) abundance; (13) year round availability; and (14) ease of

Identification.

Those species generating the highest total were placed on a short list and collection was attempted. It became apparent that ease of collection, habitat both easy to find and close to EC laboratories as well as abundance of organisms were very important criteria to be considered when selecting species for further testing. The Atlantic species selected for survival testing were: *Nephtys neotena, Nereis diversicolor, Clymenella torquata* and *Polydora cornuta*. The Pacific species selected for testing were: *Nephtys caecoides, Hediste limnicola, Axiothella rubrocincta* and *Boccardia proboscidea*. The Arctic species were *Polydora quadrilobata* and *Euchone papillosa*.

A protocol was designed for the Atlantic and Pacific species to test the candidate species for survival through field collection, handling, transporting and holding under laboratory conditions for 30 days. They were subjected to both baked and unbaked sediment and, in some cases, frozen sediment from the collection site as well as a variety of food types and rations. Results for the Atlantic species are given in Table 1.

Table 1: SURVIVAL AND WEIGHT CHANGE FOR ATLANTIC SPECIES IN CONTROL SEDIMENTS

SPECIES	% MORTALITY AFTER 30 DAYS	AVERAGE WEIGHT DIFF.* (MG)	FOOD (MG PER INDIVIDUAL)	N (ANIMALS PER REPLICATE)	SEDIMENT BAKED(+) UNBAKED(-)
P. cornuta	0%	+5.04	5	5	+
	8%	+9.27	10	5	+
N. diversicolor	0%	+39.49	8	1	+
	0%	+100.08	16	1	+
	0%	+151.91	32	1	-
C. torquata	5%	-9.45	8	4	-
	25%	-4.72	8	4	+
	40%	-5.65	16	4	+
N. neotena	8%	-0.26	5	5	-
	16%	-0.33	5	5	+
	40%	-0.29	10	5	+
	52%	-0.37	10	5	

* FINAL WEIGHT (DAY 30) MINUS INITIAL WEIGHT (DAY 0)

The results of the survival testing suggested that some species showed greater survival in unbaked sediments and baking as a sediment treatment was discontinued. It further showed that three of the species i.e. *P. cornuta, N. diversicolor* and *C. torquata* could be held for 30 days under laboratory conditions with acceptable levels of survival.

The results of similar experiments conducted on the Pacific species (Table 2) suggested that three of these species could be held in the laboratory for 30 days. The Arctic species were subjected to a combined protocol for survival and sensitivity testing. This was done because we had one opportunity for collecting Arctic species.

Table 2: SURVIVAL AND WEIGHT CHANGE FOR PACIFIC SPECIES IN CONTROL SEDIMENTS

SPECIES	% MORTALITY AFTER 28 DAYS	AVERAGE WEIGHT DIFFERENCE (mg)*	FOOD (mg PER INDIVIDUAL)	PER REPLICATES	SEDIMENT BAKED (+) UNBAKED (-) FROZEN (F)
B proboscidea	24% ** ** **	-0.02 -0.00 -0.01 +0.01	5 5 OR 10 5 OR 10 5 OR 10	N=5	+ + - F
N. caeccides	15% 24% 20% 16%	-294 -299 -284 -311	5 10 10 10	N=5	- - + F
H. limnicola	31 12.4	-3.3 -	5-8 5-8	N=1 N=1	+ -

* FINAL WEIGHT (DAY 30) MINUS INITIAL WEIGHT (DAY 0)   ** JUVENILES PRESENT

Table 3: SENSITIVITY TEST RESULTS FOR ATLANTIC SPECIES

Treatment	P. cornuta		C. torquata		N. diversicolor		N. neotena	
	1	2	1	2	1	2	1	2
WALTON (CLEAN REFERENCE)	20%	3.13-3.99	0%	10.6-15.2	20%	118.5-354.4	-	-
VANCOUVER SHIPYARD	12%	2.52-3.88	4%	9.32-14.55	10%	113.9-486.4	-	-
FALSE CREEK	24%	1.61-3.72	16%	6.48-11.31	0%	83.1-413.4	-	-
TUFT'S COVE	84%	0.84-0.91	72%	7.35-12.95	0%	117.2-410	-	-
CONTROL	8%	2.62-4.3	4%	14.96-19.74	0%	141.1-359.2	-	-
96h LC50 CADMIUM CHLORIDE (Mg/L Cd)	20		24		>92		86	

1 = % MORTALITY   2 = RANGE OF MEAN WORM WEIGHT PER JAR (mg)

Once it was established that we could hold the animals in the laboratory for the required amount of time, an experimental protocol was designed to establish the sensitivity of the candidate species through reference toxicant testing (96hr), 20 day exposure to a range of contaminated sediments, and 20 day exposure to a reference sediment. Sediment from the collection site served as the control. The results for the Atlantic species are given in Table 3, and the results for the Pacific species are given in Table 4.

Table 4: SENSITIVITY TEST RESULTS FOR PACIFIC SPECIES

Treatment	B. proboscidea		A. rubrocincta	
	1	2	1	. 2
WALTON (CLEAN REFERENCE)	4%	1.88-2.85	12%	21.1-39.8
VANCOUVER SHIPYARD	8%	2.64-4.4	32%	11.2-31.3
FALSE CREEK	16%	3.3-4.3	16%	24.6-29.1
TUFT'S COVE	76%	-	100%	-
CONTROL	0%	1.65-2.44	4%	36.0-104.7
96h LC50 Cadmium CHLORIDE (Mg/L Cd)	13		27	

1 = % MORTALITY 2 = RANGE OF MEAN WORM WEIGHT PER JAR (mg)

Of the Atlantic species, *P. cornuta* (a Spionid) and *C. torquata* (a Maldanid) proved to be the most sensitive to the test sediments and the reference toxicant. *N. neotena* did not perform well in the survival testing and was not tested in this phase, and *N. diversicolor* showed little treatment response. Of the Pacific species, *B. proboscidea* and *A. rubrocincta* belonging to the same families as *P. cornuta* and *C. torquata* respectively, proved to be sensitive to the test sediments and the reference toxicant.

The Arctic species *E. papillosa* was determined as an unsuitable test organism for sediment toxicity testing as it was a filter feeder. The spionid *P. quadrilobata* was observed to be delicate and fragmented easily, but because eggs and juveniles were observed in the tubes it was thought that this species might be suitable for culturing.

The next phase of the project was to culture the species which could be held under laboratory conditions and which showed sensitivity. An experimental design was prepared for culturing the candidate species in order to establish conditions necessary for acceptable reproduction, growth and survival. Field collections of *P. cornuta, C. torquata, B. proboscidea, A. rubrocincta* and *P. quadrilobata* were undertaken in May-June, 1994. To date, though we have been successful in holding the maldanids *C. torquata* and *A. rubrocincta* for up to five months in the laboratory, we have not achieved settlement of larvae from these two species. Better success was achieved with the spionids, especially *P. cornuta*. With this species, we have successfully determined the optimum diet for adults, juveniles and developing larvae. We have determined that under laboratory conditions they complete their life cycle in 28 days. To date, we have raised this species to the F_3 generation. Juvenile specimens have been subjected to test sediments and reference toxicants as well as to a variety of non-contaminant variables. Testing of *P. cornuta* with contaminated sediments is underway at present. This looks like a promising species for a chronic, sublethal sediment toxicity test for the Canadian ocean disposal program.

METHODS DEVELOPMENT FOR ACUTE TOXICITY TESTING USING MARINE INVERTEBRATES FROM CANADIAN COASTLINES. P. Riebel and R. Gandia. Beak

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The primary objectives of this study were: (1) to develop procedures for holding and testing arctic infaunal amphipods using Environment Canada's 10-day acute test for sediment; (2) to develop a baseline test method for acute toxicity testing of liquid phase samples using mysids from Canadian coastal areas; and (3) to compare the sensitivities of the selected species to warm water standard test organisms. Candidate test species evaluated for sediment testing (amphipods) and liquid phase testing (mysids) were *Anonyx nugax*, *Onisimus litoralis*, *Boeckosimus affinis*, *Neomysis americana* (Atlantic), *Holmesimysis costata* (Pacific), and *Mysis oculata* (Arctic). The testing program involved an extensive literature search and interviews with researchers which identified information gaps concerning each species, experiments to determine optimum holding conditions, and a round robin testing program involving three laboratories. The arctic amphipods preferred temperatures of 0-5°C, a 24 h light period and a sediment particle size <0.45 mm for *O. litoralis*, and <0.45 and >1.5 mm for *B. affinis*. Test duration experiments indicated the possibility of extending the sediment test from ten days to twenty days using *B. affinis*. Intra- and inter-laboratory test results indicalte that *B. affinis* is a good candidate species for acute whole sediment toxicity testing and has comparable sensitivity to *Rhepoxynius abronius*. *Mysis oculata* is a good candidate species for liquid phase toxicity testing, with 96 h LC_{50} results comparable to *Daphnia magna*. Control mortality in preliminary testing excluded *N. americana* and *H. costata* from inter-laboratory testing.

ECOLOGICAL RISK ASSESSMENT RISK MANAGEMENT DECISION-MAKING
SESSION CHAIR: T.A. WATSON

ASSESSING THE TOXICITY OF SURFACE WATERS DOWNSTREAM FROM A GOLD MINE USING A BATTERY OF BIOASSAYS. B.D. Wilkes[1] and J.M. Beatty Spence[2]. [1]Canadian Council of Ministers of the Environment, 326 Broadway, Suite 400, Winnipeg, MB, R3C 0S5; $_2$BC Ministry of Environment, #401-333 Victoria Street, Nelson, BC, V1L 4K3.

ABSTRACT

In 1991, a series of bioassays using fish, light emitting bacteria, crustaceans, and algae were performed in surface waters from Cascade Creek in northwest British Columbia, which received treated effluent from a gold mining and milling operation. The purpose was to assess if the resulting mixture, which contained elevated metals and cyanide, caused acute or sub-lethal toxicity based on established toxicity on rainbow trout, bacteria (Microtox test), water flea, (*Daphnia magna*), and the aquatic algae *Selenastrum capricornutum*. Multiple species bioassays were thought to assist in building a better understanding of the possible effects of the mine discharge on Cascade Creek. Sub-lethal toxicity was evident for the *Selenastrum*, suggesting that the creek water may have had the potential to reduce attached periphyton. This would affect the energy dynamics and overall productivity of the aquatic system downstream from the mine. The battery of bioassays approach proved superior for this assessment than single specie bioassays, but presented severe logistical problems and high costs when done from a remote location. In addition, problems persist in interpreting how laboratory bioassay results reflect what might actually be occurring in the receiving environment.

INTRODUCTION

In 1990, Westmin Resources re-opened the old Premier-Silbak gold mine about 20 Km north of the town of Stewart, B.C., approximately 180 Km north of Prince Rupert. The mine had been shut down for many years, much of the workings had caved in, but the company was convinced there was another 10 years of economic ore to extract from the massive sulphide deposit. The pyritic nature of the rock prompted the development of acid drainage from the old workings, with the accompanying increase in metals in surface waters draining the site. The region receives an average of 5 metres of precipitation annually, resulting in a considerable volume of drainage and all the problems associated with adequately handling those drainages at a mine site, particularly during freshet periods. The site was broken by steep mountains and deep ravines, so drainage characteristics were very complicated, with many separate flows running through the property and old workings, and accumulating in the lowermost drainage, Cascade Creek. This creek, in turn flowed to the Salmon River, just upstream of the international border, where the river entered the State of Alaska.

Because access was so difficult, a comprehensive assessment of the aquatic resources in Cascade Creek was not conducted by the company or regulatory agencies. However, as an important tributary to the Salmon River, sustaining water quality in Cascade Creek was the overall objective the company had to meet in designing it's effluent and mine water treatment facilities. Clearly, imposing tight permit limits that protected aquatic resources was a priority, due to the high fish values in the Salmon River and also because as an international watercourse, it was important to avoid a pollution incident with the Americans.

An approval to discharge liquid effluent was issued by the regional office of the provincial environment department in Smithers on November 2nd 1990, stipulating effluent limits that Westmin had to meet. Based mainly on the dilution available at the site, these limits were thought to protect the resource values downstream from contamination by heavy metals, and other contaminants such as cyanide originating from the property. Some of the key effluent limits in that November 1990 permit were:

As you can see in Table 1, the permit limits were not terribly conservative. However, since the company had great difficulty meeting these permit limits, enforcing more stringent limits would keep the company in continuous non-compliance with their permit. Over a short period of time, monitoring demonstrated that several metal concentrations were routinely higher in the discharge than the permit allowed. These included zinc (Zn), copper (Cu) and cadmium (Cd). This meant that the mixture of metal contaminants was higher in the receiving environment of Cascade Creek than was thought by regulators to be necessary to protect aquatic resources (see Table 2). The company disagreed, and asked for a permit amendment to allow the periodic higher levels of metals. The response from the regulatory agency was to require the company to demonstrate that the additional metal contamination did not threaten aquatic resources in Cascade Creek or the Salmon River. This would be done using a battery of bioassays conducted with mill effluent and creek water.

Table 1. Permit limits established in the November 1990 Westmin Mines permit compared with suggested effluent quality criteria in the BC Ministry of Environment's Pollution Control Objectives (1979).

Variable	**Permit Limits for Tailings Discharge**	**Permit Limits for Mine Water Discharge**	**Pollution Control Objectives (1979)**
Copper (diss)	0.3 mg/L	-	0.3 mg/L (max)
Zinc (diss)	1.0 mg/L	1.0 mg/L	1.0 mg/L (max)
Cyanide (tot)	1.0 mg/L	-	0.1 - 0.5 mg/L
TSS	100 mg/L	50 mg/L	25 - 75 mg/L
pH	-	6.5 - 10.5 pH units	6.5 - 10 pH units

A so-called "battery" of bioassays has been advocated in the literature as a means of making a more comprehensive assessment of the possible toxicity and environmental effect of pollution (Chapman and Long 1983). A battery of tests is superior to a single specie bioassay, since it examines potential toxicity in more than one ecosystem compartment and it can use actual receiving waters as the diluent. Bioassays cannot represent *exactly* what may happen in the receiving environment, but their results can, to some degree, verify that a given permitted level of contaminant addition is protective enough. As well, laboratory toxicity tests have been used to compliment other biological monitoring to paint a more complete picture of ecosystem impacts (Chapman 1993, Diamond et al. 1994).

Table 2. The range and average of metals levels measured at Cascade Creek downstream of the effluent and mine water discharges in 1991 compared with BC and CCME ambient water quality guidelines established for the protection of aquatic life (based on an average hardness of 46.719 mg/L $CaCO_3$).

Variable	**Cascade Creek @ Logan**	**BC Environment WQ Criteria (Pommen 1991)**	**CCME WQ Criteria (CCREM 1987)**
Cadmium (diss) mg/L	0.0002 - 0.0007 0.0003	0.0002 (max)	0.0002
Copper (tot) mg/L	0.001 - 0.041 0.0038	≤0.002 (30d ave) 0.0064 (max)	0.002
Lead (diss) mg/L	0.001 - 0.016 0.002	0.0045 (30d ave) 0.031 (max)	0.001
Zinc (tot) mg/L	0.016 - <0.005 0.043	0.03 (max) (LOEL=0.014mg/L)	0.03

METHODS

A bioassay monitoring program was developed focussing on assessing the possible acute and chronic toxicity of the mixture of metals in Cascade Creek to primary and secondary producers, as well as the usual acute test using juvenile salmonids. The timing of the program was crucial so as to capture the worst case freshet movement of contaminants off the mine site. Although the approach was not common at the time, Westmin agreed to participate in this program of bioassays, structured as follows. The monitoring program was conducted weekly over a six week period from January 29, 1991 to March 4, 1991, inclusive. Weekly samples were collected at three sites; one at the combined mine (tailings pond) discharge, another at the treated mine water pond discharge to Cascade Creek, and at a third site on Cascade Creek well downstream from the combined tailings and mine water discharges where the effect of the mine should *not* have been apparent. This site was called Cascade Creek below Logan Creek. The samples were shipped by air transport to Vancouver. There, bioassays were arranged (distributed) as follows:

Environment Canada Bioassay Lab: Microtox; 96h LC_{50} (juvenile rainbow trout, *Oncorhyncus mykiss*);

EVS Consultants: 96h LC_{50}; 96h LT_{50}; 48h Water flea (*Daphnia magna*) immobilization; 21d Water flea (*D. magna*) survival and reproduction; 96h Freshwater algae (*Selenastrum capricornutum*) cell density.

Microtox bioassays were conducted according to Microbics Corporation (1992). Rainbow trout bioassays followed methods in Environment Canada (1990a), and BC Ministry of Environment (1982). The 48 hr acute test for Daphnia followed methods described in Environment Canada (1990b) and the Alberta Environmental Centre (1990a). The 21 day Daphnia bioassay followed methods described by ASTM (1989) and the Alberta Environmental Centre (1990b). The 96 hr algal growth tests using Selenastrum capricornutum were conducted according to methods described in EPA (1989).

In addition to the company supported program, Environment Canada conducted a series of sediment bioassays using water flea (*Daphnia magna*), and BC Environment conducted long term exposure tests on yearling rainbow trout (*O. mykiss*) to determine hepatic metallothionein induction. These results will be reported elsewhere.

RESULTS

Microtox Bioassays: After reviewing three consecutive weeks of microtox test results that were non-toxic, it was agreed this component of the study should be dropped. It was clear from the results that microtox was not a useful test under these circumstances.

96 Hr Rainbow Trout LC_{50}/LT_{50}: The standard acute toxicity bioassays using rainbow trout were conducted on the combined mine discharge and the mine water discharge all six weeks of the survey. Only one sample from Cascade Creek was collected and submitted for a standard LT_{50} test. Some acute toxic response was demonstrated in the in the combined (tailings) discharge samples taken on January 29th, February 25th and March 4th 1991. The LC_{50}'s for those dates were 70%, 82% and 82% respectively. Acute toxic responses were not measured in samples collected from the combined discharge on February 4th, 11th and 18th 1991.

Samples collected at the mine water treatment pond discharge passed the LT_{50} bioassay tests on all dates sampled (ie 50% or greater of the fish survived), and on 3 of the 6 sample dates the survival was 100%. However, in samples collected on February 11th, 18th and March 4th 1991, some acute toxic response was measured with survivals of 60%, 80% and 90% respectively on those dates. One LT_{50} was conducted on a sample collected January 29th 1991, from Cascade Creek below Logan Creek. It passed the test (>50% survived) and in fact had 100% survival over the test period.

48 Hr *D. magna* EC_{50}: The purpose of these tests was to evaluate the acute toxic response of an invertebrate organism to contaminants in mine discharges from Westmin. Of the samples collected at Cascade Creek below Logan Creek all but one of the 6 had EC_{50} values greater than 100%. One sample collected at this site on January 29th had an EC_{50} of 71%. Only one of the 6 samples collected at the treated mine water pond discharge had an EC_{50} greater than 100%. In the other 5 samples, the EC_{50} ranged rom 61% to 76% (mean EC_{50} of 70%).

21 Day *D. magna* Lifecycle: The purpose of the 21 day lifecycle tests were to determine if a sub-lethal effect would be evident on *D. magna* exposed to receiving waters collected at Cascade Creek below Logan Creek. During the initial portion of the program there was some problem with the method used to conduct these tests and mortalities in the control group were greater than the acceptable 20%. This resulted in the first 4 of 6 tests conducted being rendered invalid. On the fifth week of the program the problem had been corrected and mortalities in the control group were within acceptable limits. In the last 2 chronic Daphnia tests conducted there was a slight decrease in reproduction in the sample collected February 25th and a slight decrease in survival in the March 4th sample. However, neither of these differences were statistically significant.

96 Hr Algal Growth: In these tests, the algae *S. capricornutum* was exposed to a series

of concentrations of (including undiluted or 100%) receiving water, collected at Cascade Creek below Logan Creek. The tests elicited an EC_{50} value for growth inhibition, a "no observed effects concentration" (NOEC), a "lowest observed effects concentration" (LOEC) and a percent growth inhibition or stimulation. Concurrent chemical analysis of metals and other variables in the receiving waters used in the bioassays were also conducted. The following table summarizes these results. Cascade Creek water collected below Logan Creek was diluted with laboratory control water and run in the weekly algae tests at 1.5%, 3%, 6% 13%, 25%, 50% dilutions (v/v), as well as in 100% or undiluted receiving water. In the Cascade Creek (below Logan Creek) sample collected January 29th, growth was actually stimulated at the 1.5%, 3% and 6% dilutions and inhibited in undiluted receiving waters. In all weeks of testing there was a stimulation of growth at the lowest dilutions of receiving waters. However, as seen in Table 3, in all 6 samples of 100% Cascade Creek water, growth of *S. capricornutum* was significantly and severely inhibited.

Table 3. Summary of the weekly results of *S. capricornutum* bioassays using receiving waters from Cascade Creek below mining influences, and metals concentrations in those waters from samples collected between January 29th and March 4th 1991.

Date	EC50 % (v/v)	NOEC-LOEC % (v/v)	% Inhibition in undiluted Rec Water	Tot. [Cu / Zn] mg/L Dis. [Cd / Cu / Zn] mg/L
Jan 29	65	50-100	87.8 *	[.001 / .049] [.0005 / .001 / .049]
Feb 04	50-100	50-100	81 *	[.002 / .069] [.0007 / .001 / .069]
Feb 11	75	50-100	75.9 *	[.003 / .056] [.0006 / .003 / .056]
Feb 18	50-100	50-100	54.5 *	[.003 / 0.1] [.0016 / .002 / .095]
Feb 25	50-100	50-100	77.4 *	[.002 / .058] [.0006 / .002 / .046]
Mar 04	67	50-100	91.2 *	[.001 / .079] [.0007 / .001 / .075]

* indicates the result was statistically significant.

Table 3 also shows concentrations of total copper and zinc and dissolved cadmium, copper and zinc, metals primarily thought to be imparting the impact. Comparing these metals concentrations with BC Environment ambient water quality criteria for the protection of aquatic life (Pommen 1991), minor excursions of total copper were evident in 2 samples (February 11 and 18th). Total (and dissolved) zinc levels were above the Provincial zinc criteria on all occasions. As well, dissolved cadmium was over the Provincial (total) criteria on all dates.

CONCLUSIONS

The battery of bioassays approach was able to demonstrate that contaminant levels in Cascade Creek below Logan did not cause an acute toxic response in fish (rainbow trout), invertebrates (*D. magna*), and bacteria (Microtox) exposed under laboratory conditions. However, sublethal effects were noted in both the chronic *D. magna* and *S. capricornutum* bioassays, and a decrease in survival of *D. magna* in a 48 hr EC_{50} test were noted on one occasion. The severe inhibition of the algae suggests *S. capricornutum* bioassays were the most sensitive indicators of metal toxicity. Furthermore, the *S. capricornutum* inhibition results suggest that primary production in Cascade Creek was potentially severely impaired by the contaminants from the mine. The one sample of Cascade Creek water that resulted in a *D. magna* EC50 of 71%, suggests that freshwater invertebrates may also have been effected periodically by the mine contaminants. Although these results strongly suggest that biological impacts were likely occurring in Cascade Creek, it was recognized that more detailed *in situ* biomonitoring were necessary to verify laboratory results.

Even with this in mind, amending the permit limits for metals or other contaminants upward, as the company had requested, would not have been appropriate. However, *not* amending the permit kept the company in permanent non-compliance with its legal requirements. This left the regulatory authorities with the choice of accepting that possible effects on the creek and the river were occurring, or forcing the company to comply, possibly causing it to shut down.

The battery of bioassays approach developed information that made for difficult and uncomfortable decisions on the part of regulators. In this case, Westmin was fortunate that there were no endangered species or other critical resource values at risk in Cascade Creek, as far as we know. However, resource managers must consider the realm of conclusions that may result from this type of biomonitoring and must be committed to acting in the best interest of the resource with the knowledge gained, balancing all considerations appropriately.

The results presented above, stem from very low concentrations of contaminants, suggesting that sublethal impacts in the receiving environment were likely occurring, and it was inappropriate to wait for high concentrations to appear before taking steps to more fully understand the possible effects of a discharge. The battery of bioassays approach clearly enhanced an understanding of this.

REFERENCES

Alberta Environmental Centre. 1990a. *Daphnia magna* 48 Hour Acute Toxicity Tests. *In*: Standard Operating Procedures. Volume 1, Aquatic Biology Branch, Draft Publication No. AECV90-M1, Alberta Environmental Centre, Vegreville, Alberta.

Alberta Environmental Centre. 1990b. *Daphnia magna* Chronic Static Renewal Test. *In*: Standard Operating Procedures. Volume 1, Aquatic Biology Branch, Draft Publication No. AECV90-M1, Alberta Environmental Centre, Vegreville, Alberta.

ASTM. 1989. Standard Guide For Conducting Renewal Life-cycle Toxicity Tests with Daphnia magna, Method E1193-87. *In*: 1989 Annual Book of ASTM Standards, Volume 11.04, American Society for Testing and Materials, Philadelphia, PA.

BC Ministry of Environment. 1978. Pollution Control Objectives for Mining, Smelting and Related Industries. BC Ministry of Environment, Pollution Control Branch, 1978.

BC Ministry of Environment. 1982. Provincial Guidelines and Laboratory Procedures for Measuring Acute Lethal Toxicity of Liquid Effluents to Fish. BC Ministry of

Environment Bioassay Task Force. November 1982.

CCREM. 1987. Canadian Water Quality Guidelines. Prepared for the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers. March 1987.

Chapman, P.M. 1993. The role of ecotoxicology in environmental impact assessment (EIA). The Environ. Professional 14: 139-144.

Chapman, P.M., and E.R. Long. 1983. The use of bioassays as part of a comprehensive approach to marine pollution assessment. Mar. Poll. Bull. 14: 81-84.

Diamond, J.M., J.C. Hall, M.P. Dudley, and D. Gruber. 1994. Use of an integrated monitoring approach to determine site-specific effluent metal limits. Water Environ. Res. 66: 733-743.

Environment Canada. 1990a. Biological Test Method: Acute Lethality Test Using Rainbow Trout. Environmental Protection Series, Report EPS 1/RM/9, July 1990.

Environment Canada. 1990b. Biological Test Method: Acute Lethality Test Using Daphnia spp. Environmental Protection Series, Report 1/RM/11, July 1990.

EPA. 1989. Short-term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms, 2nd Edition. US Environmental Protection Agency, Cincinnati, OH. EPA/600/4-89/001.

Microbics Corporation. 1992. Microtox Update Manual. December 1992.

Pommen, L.W. 1991. Approved and Working Criteria for Water Quality. Ministry of Environment, Water Management Division, Water Quality Branch, Victoria B.C. May 1991.

CANADIAN ENVIRONMENTAL QUALITY GUIDELINES FOR DIOXINS AND FURANS - A MULTIMEDIA APPROACH. D.E. Andersen[1], H. Singleton[2], D. Spry[3], S.L. Walker[1], K.A. Keenleyside[1], R.A. Kent[1], and D. MacDonald[4]. [1]Evaluation and Interpretation Branch, Environment Canada, Ottawa, ON, K1A 0H3; [2]BC Ministry of Environment, Victoria, BC, V8V 1X5; [3]Ontario Ministry of Environment and Energy, Toronto, ON, M4V 1P5; [4]MacDonald Environmental Sciences Ltd., Ladysmith, BC, V0R 2E0.

Environmental quality guidelines are used by Canadian provincial, territorial and federal agencies to assess environmental quality problems and to manage competing uses of resources. A literature review was conducted on the environmental concentrations, physical and chemical properties, environmental fate and persistence, bioaccumulation potential, toxicity to freshwater and marine biota, and toxicity to non-target crops and livestock of polychlorinated dibenzo-*p*-dioxins (dioxins) and polychlorinated dibenzofurans (furans). This information was used in conjunction with published, nationally approved, protocols to derive Canadian water quality, sediment quality, and tissue residue guidelines. Water quality guidelines are recommended for the protection of aquatic life and irrigation water. Interim freshwater and marine sediment quality guidelines are recommended for the protection of aquatic life and wildlife. Furthermore, tissue residue guidelines are recommended for the protection of both freshwater and marine wildlife consumers of contaminated aquatic biota. Guidelines for several other uses of the aquatic ecosystem could not be developed due to insufficient data.

MCPA - AN OVERVIEW OF ITS USE AND ENVIRONMENTAL IMPACTS IN CANADA.

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MCPA is one of the top five pesticides used in Canada. It is a systemic herbicide used to control a wide spectrum of broadleaf weeds in agricultural and non-crop land applications. It has the potential to contaminate aquatic resources through a variety of transport routes. It is relatively soluble in water and slightly persistent in soils. As it enters the different environmental compartments, it does not necessarily contaminate them. The current challenge is to assess the potential impact of MCPA in the environment. In terms of water quality, potential impacts are apparent when the compound is present in the water used for a specific purpose such as irrigation or livestock watering. The Canadian Water Quality Guidelines have been designed to provide a water quality management tool to ensure that agriculture through the use of pesticides does not degrade Canadian freshwater and does not harm crops or livestock. This presentation will review the uses and impacts of MCPA in Canada.

CONTAMINATED GROUNDWATER - THE NEGLECTED CHAPTER IN ENVIRONMENTAL ASSESSMENTS.

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The environmental effects assessment of priority substances under CEPA requires a holistic, ecosystem approach in which the concentrations and fate of the substance in each environmental compartment, and its direct and indirect effects on biota, must be considered. The groundwater compartment, historically, has received only cursory attention in environmental effects assessments and risk management. However, a proper assessment and risk management should consider this compartment. The evaluation and use of Canadian groundwater data in environmental assessments poses several challenges including: (1) lack of accessible data on the extent of groundwater, and on the nature and severity of its contamination; (2) lack of knowledge of groundwater ecosystems and on effects of contaminants on these ecosystems; (3) scarcity of studies documenting discharge of contaminated groundwaters to surface; and (4) lack of data on specific impacts of such discharges on major water uses (aquatic life, drinking water, agriculture, *etc.*). Nonetheless, two priority substances (tetrachloroethylene and trichloroethylene) were declared as *"CEPA toxic"*, in part due to the observed surface discharge of contaminated groundwater at concentrations exceeding toxic effects thresholds. This presentation will explore the issue of groundwater contamination with respect to the environmental risk assessments of trichloroethylene and tetrachloroethylene. The potential impacts of contaminated groundwater on various water uses (irrigation, livestock watering, surface water recharge and wildlife watering) will be discussed.

CHLORINE AND ORGANOCHLORINES IN THE ENVIRONMENT: A PERSPECTIVE

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"Chlorine bad, no chlorine good; therefore, ban chlorine" is the essence of a popular argument concerning chlorine and chlorinated organics in the environment. In reality every problem is a risk and every solution itself represents some risk. There are no risk free solutions. It is only possible to achieve a balance of risk, benefit, and economic cost, a process formally termed risk cost-benefit analysis. The objective is to ensure that the adopted solution is less risky than the original problem. The chlorine debate needs an impartial examination if a clear perspective is to be communicated to interested parties. Three main areas are examined: (1) chemical and toxicological information; (2) the general process of making judgements; and (3) the consequences of judgements made. A number of toxicological aspects and concerns are reviewed e.g., chlorine and organochlorines are artificial and unnatural, all organochlorines are persistent and bioaccumulative, all organochlorines are toxicologically undesirable and should be banned, and chlorinated organics are more toxic than non-chlorinated organics. The scientific data are placed within the context of society's general judgement processes, using a quasi-legal framework. The consequences of judgements made examines a possible ban of chlorine in water disinfection with a current example. Although directed towards the use of chlorine in water disinfection, much of the information is applicable to the broader debate of the environmental significance of organochlorines and other chemicals.

RESEARCH AND APPLIED INVESTIGATION TO ESTABLISH A PENTACHLOROPHENOL (PCP) CONCENTRATION TO PROTECT A SALMON CREEK IMPACTED BY WOOD TREATMENT OPERATIONS.

T.A. Watson[1], K.L. Cooper[1], M.G. Rankin[2] and E. Garay[3]. [1]Triton Environmental Consultants Ltd., Richmond, BC, V6V 2L1; [2]Golder Associates Ltd., Burnaby, BC, V6K 2W1; [3]Bell Pole Co. Ltd., Lumby, BC.

The use of pentachlorophenol (PCP) at a utility pole treatment operation over a 25 year period resulted in the contamination of soils, groundwater and an adjacent stream inhabited by salmonids. Monitoring of PCP in resident fish and caged leeches indicated that PCP levels were higher downstream of the treatment site. The objective of the present study was to evaluate the significance of elevated PCP levels in biological receptors and to develop a PCP concentration that would protect fish over the long term. First steps in development of a protection level included a literature review of PCP toxicity and bioaccumulation in aquatic organisms. These data were subsequently conpared with the PCP guideline levels proposed by the United States Environmental Protection Agency (EPA). *In situ* studies using caged rainbow trout combined with additional monitoring of other receptors indicated that a PCP concentration of 0.44 ug/L in the creek would protect fish and would not result in accumulation of PCP above 100 ng/g. This protection level is more than 3 times lower than the lowest no observed effect concentration (NOEC) of 1.74 ug/L for salmonids and is higher than site specific provisional objectives proposed by regulatory agencies. Data from this investigation are being used, in part, as a basis for establishing appropriate remediation levels in soil and groundwater by application of ecological risk assessment.

APPLICATION OF ECOLOGICAL RISK ASSESSMENT FOR SOIL REMEDIATION AT A CHLOROPHENOL TREATMENT OPERATION. M.G. Rankin[1], D. Chorley[1], R. Zapf-Gilje[1], T. Watson[2], K.L. Cooper[2] and E. Garay[3]. [1]Golder Associates Ltd., Burnaby, BC, V6K 2W1; [2]Triton Environmental Inc., Richmond, BC, V6V 2L1; [3]Bell Pole Co. Ltd., Lumby, BC.

A former B.C. wood treatment operation engaged in the production and treatment of utility poles has resulted in chlorophenols (mainly pentachlorophenol) contamination in soil and groundwater associated with the site. While these contaminants have the potential to enter a salmon-bearing stream adjacent to the property, a groundwater interception trench presently renders the exposure pathway inoperable. The risk management goal is to remediate the contaminated soil (source) to a level which will yield acceptable aquatic contaminant concentrations vis a vis ecological risks, without the need for groundwater interception. Ecological risk assessment principles are being employed to develop site-specific target clean-up criteria for soil. The presentation will highlight the methodology being employed for derivation of hazard quotients, including a probabilistic contaminant transport and food chain model for exposure assessment, and development of a probability distribution function for the toxicity reference values.

MICRO-SCALE BIOTESTS IN TOXICITY ASSESSMENTS
SESSION CO-CHAIRS: G.L. GILRON AND D.H. LYNN

A BACTERIAL TOXICITY ASSAY PERFORMED WITH MICROPLATES, MICROLUMINOMETRY AND MICROTOX^{R} REAGENT. C. Blaise[1], R. Forghani[1], R. Legault[1], J. Guzzo[2] and M.S. Dubow[2]. [1]Centre Saint-Laurent, Environnement Canada, 105 rue McGill, Montréal, QC, H2Y 2E7; [2]Department of Microbiology and Immunology, McGill University, 3775 rue Université, Montréal, QC, H3A 2B4.

We have developed a procedure for undertaking a Microtox^{R}-based test by coupling microplate and microluminometric technologies. Sample dilutions are prepared in a 96-well polystyrene microplate kept at 15°C, while Microtox^{R} reagent and diluent are placed in an opaque microluminometry-compatible 96-well microplate equally kept at 15°C. Exposure begins when sample aliquots are brought into contact with bacterial reagent in the opaque microplate. After specific exposure times (5-, 15-, 30- and 60-min), bacterial luminescence is rapidly measure by placing the opaque microplate in a microluminometer. Reproducibility of the procedure, as well as general agreement of EC50 end-point values with published reports, was demonstrated after toxicity trials with six metals (Ni^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+} and Cr^{6+}). Results suggest that a 60-min exposure time may have value in getting more "sensitivity mileage" out of this Microtox^{R}-based assay. This microplate procedure possesses attractive features which augment sample throughput and information output. Further refinement and validation studies are on-going in our laboratories.

DEVELOPMENT AND VALIDATION OF A NEW, RAPID AND ECONOMICAL PROTOZOAN BIOASSAY FOR PULP AND PAPER EFFLUENTS: II. INTERLABORATORY COMPARISON. G.L. Gilron[1], D.H. Lynn[2], S.G. Gransden[2] and R.P. Scroggins[3]. [1]Beak Consultants Limited, 14 Abacus Road, Brampton, ON, L6T 5B7;. [2]Microvita Consulting, 23 Lyon Avenue, Guelph, ON, N1H 5C5; [3]Technology Development Branch, Environment Canada, 4th Floor, Place Cartier, 425 St. Joseph Blvd., Hull, QC, K1A 0H3.

This study is part of an ongoing research program aimed at the development of a standard protozoan bioassay to complement Environment Canada's Biological Test Method Development program. To date, the study has focused on the development of a sublethal behavioural bioassay using ciliates as test organisms, utilizing the chemotactic response exhibited by ciliates when they are exposed to environmental toxicants. During the previous phases, a protocol was developed and tested with several reference toxicants, and with various industrial effluents; subsequently, the protocol was technically refined, including enhancements to cell enumeration methods, pretreatment of cell cultures to sensitize organisms to toxicants, and the variation of test exposure time. This phase of the study involved an exploration of the quality assurance of the test protocol to evaluate the reproducibility, clarity, and comprehensibility of the standard operating procedure (SOP) and data resulting from it, through an interlaboratory calibration exercise (i.e., "round-robin"). Participating labs were sent a standard operating procedure (SOP), a full set of test equipment, standard reference toxicants (NaCl, $CdCl_2$ and guaiacol) and cell cultures. The labs then conducted a full series of tests with reference toxicants and whole effluents. Results were then compiled, tabulated, and statistically analyzed. The results from the calibration exercise and the implications for the validity and reproducibility of the SOP will be discussed.

THE USE OF BACTERIAL BIOASSAYS FOR MEASUREMENT OF TOXICITY IN SEDIMENTS. R. Lifshitz[1] and K.K. Kwan[2]. [1]Environmental Biodetection Products, Inc., Brampton, ON, L6T 5B7; and [2]Environment Canada, National Water Research Institute, Burlington, ON, L7R 4A6.

Two rapid bacterial bioassays, the Sediment-Chromotest and Microtox Solid-Phase Procedure (SPT) were used to measure the acute toxicity of sediments and of solid waste samples. Both assays enable the toxicity measurement of bioavailable compounds in solids, dierctly without extraction, and both bioassays are rapid and easy to use. The response measured in the Sediment-Chromotest was in inhibition of β-galactosidase enzyme biosynthesis of *E. coli*, while the response measured in the Microtox SPT was the inhibition of bioluminescence in *Photobacterium phosphoreum*. A comparative evaluation revealed that both bacterial bioassays strongly correlated with each other, and both were sensitive, consistent and reliable.

TOXICITY ASSESSMENT OF THE PHOTODEGRADATION PRODUCTS OF ANTHRACENE, PHENANTHRENE AND FLUORANTHENE USING *Photobacterium phosphorium*. B.J. McConkey, D.G. Dixon and B.M. Greenberg. Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

Light, particularly UV radiation, has been shown to increase polycyclic aromatic hydrocarbon (PAH) toxicity, through photosensitization reactions (production of singlet oxygen) and photomodification of chemicals to new species. The most common form of photomodification is oxidation, producing quinones, diols and carboxylic acids. Our work has shown that PAHs modified by solar radiation can be more toxic than intact PAHs. We have thus been identifying the products of photomodification for several PAHs. The products formed were isolated by HPLC, and identified by GC/MSD, NMR, and comparison with chemical standards. The results reveal a complex array of photoproducts, with at least 10 compounds from each PAH tested. Acute toxicity of the degradation products was assessed using *Photobacterium phosphorium*, a marine bacteria. As this bacteria emits light as a natural byproduct of enzymatic function, the reduction of light emission can be used as a toxic endpoint. This assay is useful in screening large numbers of compounds for their potential toxicity to higher organisms. The effects of photomodified PAHs in the natural environment has not been thoroughly investigated. As these products exhibit toxic effects, and are not monitored as potential toxicants in the environment, it is important to include the toxicity of these products in determining the long term effects of PAH impacts.

ENVIRONMENTAL ASSESSMENT OF A DREDGED MATERIAL DUMP SITE USING MULTITROPHIC LEVEL SEDIMENT BIOASSAYS AND A BIOACCUMULATION TEST.

[1]K.L. Tay, [1]K.G. Doe, [1]A.J. MacDonald, [1]S.J. Wade, [1]J.D.A. Vaughan, [1]A.L. Huybers, [1]G.D. Wohlgeschaffen, [2]K. Lee and [2]R. Laroque. [1]Environment Canada, 45 Alderney Drive, Dartmouth, NS, B2Y 2N6; [2]Fisheries and Oceans, Institute Maurice Lamontagne, Mont-Joli, QC, G5H 3Z4.

A three year environmental assessment of sediments at the dredged material dump site outside of Saint John Harbour, New Brunswick was conducted from 1992 to 1994. The toxicity of the dump site sediments was evaluated using a battery of four bioassays based on: the luminescence of *Photobacterium phosphoreum* (Microtox™ pore water and solid-phase); indigenous bacteria exoenzyme activity (MCA-Leucine); amphipod survival (whole sediment assays, *Amphiporeia virginiana* and *Rhepoxynius abronius*); and sea urchin fertilization success (pore water assay, *Lytechinus pictus*). In addition, sediment samples were analyzed for metals, PAHs, PCBs, organic carbon, grain size, sulphide, and ammonia in sediment pore water. The uptake of sediment contaminants was assessed by chemical analyses performed on the digestive glands and muscular tissues of lobster (*Homarus americanus*) collected from the dump site. The results of the study were used to evaluate the selected bioassays for assessing the potential for long-term biological effects from the disposal of dredged materials at ocean dump sites.

Two bacterial tests, Microtox™ solid-phase and exoenzyme (MCA-Leucine), were the most sensitive to the dump site sediments with toxic effects observed in 14 of 19 samples and 18 of 19 samples respectively. The other assays in order of decreasing sensitivity were sea urchin (*L. pictus*) fertilization, amphipod (*A. virginiana* and *R. abronius)* and Microtox™ pore water.

Heavy metal concentrations were low in all dump site sediment samples. PCB levels were generally below detectable levels. PAH concentrations ranged from less than detectable to a maximum of 13.85 mg/kg. No significant correlation were found between concentrations of PAH or PCB and the measured endpoints of the three most sensitive bioassays. However, significant correlations were detected between those same endpoints and concentrations of ammonia in sediment pore water and sediment sulphides.

PAH concentrations in the digestive gland and muscular tissue samples of lobster collected from the disposal site were low in comparison to tissue concentrations from lobster taken from other harbours in industrialized areas of Atlantic Canada.

Based on the toxicity tests and the bioaccumulation survey, it is concluded that the toxicity of the dump site sediment is most likely due to the presence of ammonia and/or sulphides in the sediment samples. The concentrations of PAH in some of the dump site sediments are higher than the ocean disposal regulated limit (2.5 mg/kg); however, no significant correlations were observed between the sediment PAH and the toxicity endpoints. Further, no significant uptake of PAH was observed in the digestive glands and muscular tissue samples of the lobsters caught at the disposal site.

The sensitivities of the bioassay tests and their environmental relevance are a critical considerations in regulatory decision-making relating to the ocean disposal of dredged materials. Based on the data from this and other studies, the following criteria are recommended in for use in the interpretation of sediment toxicity test results. Microtox™ solid-phase assay: sediments producing a 5 minute IC_{50} less than 1000 ppm are toxic. Amphipod test (*R. abronius* and *A. virginiana)*: sediments are toxic if mortality is at least 20 percent higher than reference sample mortality and statistically different. Sea urchin fertilization (*L. pictus*): sediments are toxic if fertilization is at least 25 percent lower than control sample fertilization and statistically different. Indigenous bacteria exoenzyme test: sediments are toxic if control site activity is reduced by 20 per cent or more of the normalized dilution value at a test sediment concentration of 25 per cent. Further investigation is recommended for the selection of a more sensitive endpoint for the Microtox™ pore water assay.

TOXICOLOGY IN AQUATIC ORGANISMS
SESSION CHAIR: M.A. LINES

BEHAVIOURAL AND ELECTROPHYSIOLOGICAL EVIDENCE THAT OLFACTION MEDIATES AVOIDANCE OF CADMIUM MY LAKE WHITEFISH (*Coregonus clupeaformis*). R.E. McNicol and T.J. Hara. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB, R3T 2N6.

Fish will avoid contact with a variety of pollutants; yet little is known of the chemosensory mechanisms mediating avoidance behaviour. While some studies have measured olfactory and gustatory responses to contaminants, few have investigated responses at environmentally realistic concentrations, or attempted to correlate chemosensory responses with avoidance behaviour. Our study investigated whether avoidance of cadmium by lake whitefish (*Coregonus clupeaformis*) is mediated through olfaction or gustation, by using a combination of behavioural and electrophysiological measures. Whitefish displayed a bimodal concentration-avoidance response relationship, avoiding at 1 and at ≥25 µg/L, but showing a neutral response at 5 µg/L. Anosmic fish did not avoid cadmium at the test concentration (0.1-250 µg/L). Perfusing the olfactory rosettes with cadmium solutions produced positive electroolfactograms (EOG) responses for concentrations ≥ 5 µg/L; responses at lower concentrations were inconsistent. Microelectrode recordings indicated that cadmium triggered increased sensory neuron activity in the olfactory epithelium. Attempts to record gustatory responses to cadmium or standard stimulants failed. Thus, while detection of cadmium by gustation cannot be ruled out, our results indicate that avoidance of cadmium by whitefish is mediated by olfaction.

INTERPRETATION OF DATA FROM SHORT-TERM GROWTH TESTS USING THE SACFRY TO EARLY SWIM-UP LIFE STAGE OF RAINBOW TROUT. C. Neville. Ontario Ministry of Environment and Energy, Toronto, ON, M9P 3V6.

I have developed a short-term, rainbow trout early-life-stage growth test to provide data on individual chemicals for the development of Federal and Provincial Water Quality Guidelines to protect salmonids, and to monitor possible chronic toxicity effects in effluents and receiving waters that are not acutely toxic. Since it is never possible to accurately predict chronic effects in the environment from laboratory data, there will always be some degree of uncertainty, so for practical purposes I had to develop the most cost effective test that had the least degree of uncertainty. The test had to be as short, simple, and sensitive as possible to minimise costs and to detect low levels of toxicity. It also had to detect non-specific chronic effects, and the measured effect had to be known to reduce the health and life expectancy of populations as well as individual organisms. I used the late sacfry to early swim-up life stage to obtain the shortest period for optimal uptake of toxicants as well as expression of effects, and growth as the measured effect. Sublethal test concentrations were used to avoid mortality.

Wet weight gains in individually weighed and exposed fish were used to assess growth of rainbow trout. Individual weight gain has to be used to measure growth, rather than the final weight, since even sibling sacfry, hatched on the same day, have highly variable weights. The initial weights can be measured in ≈ 11 d old sacfry, when the larva is robust and the yolksac and oil globules are still large. Dry weights cannot be used for growth at this lifestage since there is a loss of dry weight during sacfry development. Not all of the energy from the metabolised yolk can be used for larval growth and development - some has to be used for respiration and activity. Hence, before exogenous feeding, if dry weights are used, any toxicant that has slowed the rate of yolksac absorption in a test group will appear to have produced a higher growth rate than the controls. This can still confuse establishing significant differences from the controls in the swim-up fry dry weights during the first few days of exogenous feeding, if feeding is moderately delayed or diminished. (The increase in wet weight during sacfry development is due to water absorption into the larva, induced by the absorbed yolk).

A static with 24 h renewal exposure system was used. Temperature, pH, and O_2 saturation were monitored in the pre and post 24 h exposure solutions. Any abnormal activity was noted throughout the test. Feeding for 5 days was initiated at the early swim-up stage; age at onset of feeding, and amount consumed compared to the controls were noted. The fish were not fed on the final day. After measuring the final individual wet weights, at ≈ 23 days old when yolksac absorption was complete in the controls, any visible abnormalities compared to the controls were noted. The degree of yolksac absorption, measured as the width in mm of the gap in the epithelium covering the yolksac, (0 mm = complete, >2 mm = severe delay), was also recorded. Dry weights were also measured if abnormal feeding and swollen tissues were noted, and the individual fish % water content was calculated from the wet and dry weights to identify edema, since this would invalidate the wet weight gains. However, edema should rarely occur in this test using sublethal concentrations, as it usually results in acute mortality rather than sublethal, chronic effects.

Dose/response curves, using measured test concentrations, group mean wet weight gains (not transformed), and hypothesis tests were used to interpret the data, together with the notes on feeding, appearance, and ambient chemical parameters. Tests have been carried out with sublethal concentrations of copper, and of 2,4,5-trichlorophenol, and 3,4-dichloroaniline by N. Karrow, University of Waterloo. In the copper and TCP tests there was no difficulty in interpretation even though the dose/response curves showed a non-linear response in concentrations close to the LOEC, indicating that the fish could temporarily compensate for small, gradually developing metabolic changes caused by the toxicant. This response is often seen in sensitive growth tests. The LOEC for copper was 12 µg/L. This concentration caused 30% growth reduction in a 47 day rainbow trout eyed egg to early juvenile test by McKim et al. in 1978, (Bull. Environ. Contam. Toxicol. 19: 608-616), but under the experimental conditions at that time, it was not found to be significant. The dose/response curve steepened sharply between groups 5 and 6 in the TCP test because at 438 µg/L, (group 6), there was no exogenous feeding and the fish lost weight during the test. The LOEC was <34 µg/L. No ELS growth tests using rainbow trout could be used for comparison, but it was 20 times more sensitive than a 7 d fathead minnow growth test reported by Norberg-King in 1989, (Environ. Toxicol. Chem. 8:1075-1089).

Interpretation of the DCA test was not quite so simple. The dose/response curve flattened from groups 4 to 6, indicating a change in toxic mechanism in groups 5 and 6. In group 6, yolksac absorption was severely delayed, the fish did not eat, were swollen, and the % water content was significantly increased, indicating edema. The wet weight data from this group were discarded. These effects were evident in half the group 5 fish, but were less severe, and the % water content was not significantly increased. Most fish ate very little. Nevertheless, the wet weight gain was similar to the group 6 fish. It was evident that in this group the decrease in % water content caused by delayed yolksac absorption was balanced by the less severe edema. Thus, in this group, the % water content could not be used to identify edema but it could be identified by the flattened dose/response curve combined with almost no feeding, therefore the group 5 data were also discarded. Group 4 (the LOEC) began showing moderate effects and group 3 slight effects by the 4th day of feeding. The LOEC and NOEC were 139 and 55 µg/L respectively. The sensitivity of this test was approximately twice that of a 14 day growth test using 1-3 g early juvenile rainbow trout, developed by Crossland and reported in 1990, (Chemosphere, 21:35-40), (LOEC = 210 µg/L, NOEC = 120 µg/L). However, when Crossland extended his test to 28 days, the LOEC and NOEC were reduced to 39 and 19 µg/L, respectively. The reduction of feeding evident by the 4th day in the LOEC and NOEC groups of this sacfry to swim-up fry test indicate that extending the exposure period for 1-2 weeks would also lower the LOEC and NOEC. Other tests using DCA have shown that extension of the exposure period from 1 to 2 or 3 months had little effect on the LOEC.

These results show the sensitivity of this cost effective, short-term, sublethal, sub-chronic, rainbow trout early-life-stage growth test, using the late sacfry to early swim-up life stage. It also leaves no doubt about the greater sensitivity of growth compared to mortality for estimating chronic levels of toxicity.

SUBSTITUTED PHENANTHRENES AS INDUCERS OF HEPATIC MIXED FUNCTION OXYGENASE (MFO) IN RAINBOW TROUT (*Oncorhynchus mykiss*). J.L. Parrott[1], B.K. Burnison[1], P.V. Hodson[1], M.E. Comba[1] and M.E. Fox[2]. [1]Aquatic Ecosystem Conservation Branch and [2]Aquatic Ecosystem Restoration Branch, Environment Canada, Burlington, ON, L7R 4A6.

In the search for compounds responsible for MFO induction at pulp mill sites we compiled a database of structures of chemicals associated with effluents and compared these to structures of known inducers. Phenanthrene-type compounds were one of the groups of chemicals we tested for ability to induce MFO in rainbow trout. Four-day exposures to retene (100 µg/L), and 3,6-dimethylphenanthrene (100 µg/L) induced hepatic MFO activity, while exposure to phenanthrene (up to 1000 µg/L) did not. The retene tested was only 87.7% pure and several of the impurities were identified as methyl to butyl-substituted phenanthrenes, phthalates and tetrahydroretene. Tests of solid phase extraction fractions of retene showed induction in the more hydrophobic fraction (containing retene), however tests of pure retene (99%) suggested it was not the primary cause of the induction. The inducer(s) appears to be a contaminant, either a substituted phenanthrene or tetrahydroretene. From a structure-activity point of view, these findings suggest that addition of a methyl or other small, two to three-carbon group to phenanthrene produces a structure that may induce MFO activity in rainbow trout.

THE IMPORTANCE OF A GRADIENT AS A CUE FOR FISH THAT AVOID TOXICANTS AND WHOLE EFFLUENTS. G.F. Westlake, L.J. Novak and K.M. Murphy. Ontario Ministry of Environment and Energy, Etobicoke, ON, M9P 3V6.

This is about the cues fish use to detect toxicants. We compared the responses of rainbow trout which were immersed in an constant concentration during observation with responses when the fish were exposed to a concentration gradient throughout observation. The fish responded to a number of substances and some whole pulp and paper mill effluent samples differently depending on which exposure method was used. The trout did not respond to copper unless there was a spatial gradient or the concentration was changed often. This implies that fish may not always be able to detect shallow gradients or that they have a different, much more subtle response mechanism than the one they use in steep gradients. The detection threshold in the spatial gradient was estimated at 23.5 µg/L for copper. During toxicant exposure, fish were observed to make violent head movements which appeared to be a type of cough and may be involved in the mechanism of the response.

TOXICOLOGY ASSESSMENTS IN LARGE RIVER SYSTEMS
SESSION CHAIR: S. THORNLEY

CHEMICAL CONTAMINATION AND ULCERATIVE LESIONS IN ATLANTIC TOMCOD (*Microgadus tomcod*) FROM THE ST.LAWRENCE RIVER. Y. de Lafontaine[1], Y. Mailhot[2], S. Lair[3], D. Martineau[3] and L. Berthiaume[4]. [1]Centre Saint-Laurent, Environnement Canada, Montréal, QC, H2Y 2E7; [2]Ministère Environnement et Faune, Trois-Rivières Ouest, PQ, G8Z 4L7; [3]Université du Montréal, Faculté de Médecine Vétérinaire, St-Hyacinthe, QC, [4]Institut Armand-Frappier, Laval, QC, H7N 4Z3.

The prevalence of oral and body ulcers in anadromous Atlantic tomcod (*Microgadus tomcod*) from the St.Lawrence upper estuary has promoted the investigation of the nature and levels of contaminants present in this fish species. Causes of ulcers observed in tomcod are unknown, and no evidence of viral infection was found. Using a factorial experimental design, 40 fish with ulcerative lesions were compared with 40 control fish showing no external evidence of disease. Both inorganic and organic contaminants were analysed in 3 body parts: liver, gonads and carcass. Mercury and arsenic were frequently detected in high concentrations but cadmium and lead were only rarely present. Most commonly detected organic compounds include PCB, DDT derivatives, mirex and hexachlorobenzene. These compounds are similar to those found in other fish species from the upstream freshwater portion of the St.Lawrence. Levels of contaminants in tomcod vary significantly between body parts and very high concentrations were measured in both liver and gonads. Fish with ulcers have significantly lower gonado-somatic index (GSI) and higher hepato-somatic index (HSI) revealing a poorer fish condition. Taking the effects of fish size and age into account, no significant difference in contaminants concentrations was found between fish with ulcers and control fish.

ASSESSMENT OF DETROIT RIVER SEDIMENTS AND BENTHIC MACROINVERTEBRATE COMMUNITIES - 1991. D.G. Farara and A.J. Burt. Beak Consultants Limited, 14 Abacus Rd., Brampton, ON, L6T 5B7.

In 1991, benthic macroinvertebrate and sediment samples were collected at 77 sites located along the Detroit River. The study objectives were to document spatial and temporal trends in the environmental condition of the river since 1980. Sediment toxicity tests using fathead minnows, *Chironomus tentans* and *Hexagenia limbata* were undertaken on samples collected at nine sites. Cluster analysis and community ordination identified five distinct macroinvertebrate communities which were used to delineate zones that were slightly to severely impacted. The most extensive areas of severely impacted benthos and severely contaminated sediments were documented along the American shoreline from the Rouge River to the southern end of Grosse Ile. Discriminant analysis indicated that higher concentrations of PAHs, solvent extractables, PCBs, and heavy metals were important in structuring benthic communities. Acute toxicity data generally supported delineation of zones of severely impacted benthic macroinvertebrate communities and severely contaminated sediments. Furthermore, sublethal toxicity data supported the delineation of moderately impacted benthic macroinvertebrate communities and moderately contaminated sediments. Overall, the impacted areas did not decrease in size since 1980 and there is evidence, based on benthic macroinvertebrate community structure and sediment quality, to suggest that the environmental condition of the Detroit River has deteriorated since 1980.

CONNECTICUT RIVER COAL TAR TOXICITY FOR SHORTNOSE STURGEON (*Acipenser brevirostrum*) EMBRYOS AND LARVAE. R.M. Kocan[1], M.B. Matta[2] and S. Salizar[3]. [1]University of Washington (HF-15), Seattle, WA, 98195; [2]NOAA/HAZMET, 7600 Sand Point Way, Seattle, WA, 98115; [3]EVS Consultants, 2517 Eastlake E, Seattle, WA, 98119.

Coal tar contaminated sediments collected from the Connecticut River near Holyoke, MA, were shown to be toxic to shortnose sturgeon embryos and larvae in both whole sediment flow-through and coal tar elutriate static-renewal laboratory toxicity tests. Forty-eight hour old embryos produced by a single female shortnose sturgeon collected in the Connecticut River were used for exposure to coal tar contaminated sediments. Embryos were exposed by placing them on the surface of sediments which had been layered on the bottom of two-liter vessels and incubating them in flowing dechlorinated city water for 21 days. Exposure of embryos and larvae to clean laboratory sand and uncontaminated Connecticut River sand collected upstream of coal tar deposits, resulted in no significant differences in embryo-larval survival, while coal tar-contaminated sediment resulted in greater than 95% combined embryo-larval mortality after 21 days exposure in a flow-through system. The primary mechanism for exposure appeared to be via direct contact with contaminated sediment rather than via soluble hydrocarbon exposure in the flow-through exposure system. When the sturgeon embryos were exposed to an aqueous extract of coal tar, both embryo and larval mortality significantly increased at hydrocarbon concentrations greater than 0.19 mg/L (10%) of the water soluble fraction, with 100% mortality observed at and above 50% of the water soluble fraction. The significance of these findings is that petroleum hydrocarbons can retain at least some toxic components after long periods of "weathering" and exposure to flowing water. The last known deposition of coal tar into the Connecticut River occurred at least 50 years ago, yet it retained enough toxicity to kill developing sturgeon embryos that were in direct contact with it.

PRELIMINARY MERCURY IN FISH STUDY RESULTS FROM THE OLDMAN DAM RESERVOIR. J.W. Moore, S. Wu, L.Z. Florence, K.L. Smiley and D.S. Lucyk. Alberta Environmental Centre, Bag 4000, Vegreville, AB, T9C 1T4.

In 1991, the Alberta Environmental Centre (AEC) initiated a five year research study project on mercury in fish inhabitating the newly formed Oldman River Dam Reservoir and the rivers within the Oldman River drainage basin. This project is part of a fisheries mitigation strategy for the basin that requires no net loss of fish habitat or recreational fishing opportunities, consistent with the Province of Alberta's policy of sustainable development. The impetus for this project is the possibility that mercury concentrations in fish might increase after impoundment and exceed federal human health consumption guidelines (0.5 mg/kg). Increases in mercury in fish have been noted in several reservoirs in Canada and elsewhere. Since the rate of methylation and the uptake of mercury by fish and other aquatic species depends on a number of factors, the extent of mercury accumulation in fish in this new reservoir requires observation and study. Preliminary results on the project objectives to assess the changes in the concentration of mercury in the muscle tissue of fish over a five year period and some supplementary fish population inventory results in the reservoir and surrounding rivers will be presented. Based upon the 1991, 1992 and the preliminary 1993 evaluations, mercury residues in fish from the reservoir and the surrounding rivers pose no health threat to human consumers of fish.

BIOLOGICAL EFFECTS OF CONTAMINATED SEDIMENTS FROM THE ST. CLAIR AND ST. MARYS RIVERS IN LABORATORY BIOASSAYS. D. Bedard[1], S. Petro[1] and P.B. Kauss[2]. [1]Standards Development Branch, [2]Environmental Monitoring and Reporting Branch, Ontario Ministry of Environment and Energy, 125 Resources Road, Etobicoke, ON, M9P 3V6.

Sediments in areas of the St. Clair River and the St. Marys River contain concentrations of mercury, organochlorine compounds such as hexachlorobenzene and polychlorinated biphenyls, or of polycyclic aromatic hydrocarbons (PAHs) in exceedence of the Ontario Provincial Sediment Quality "Severe Effect Level" Guidelines. At these levels, the sediment is considered "grossly polluted" and has the potential to "significantly affect use of sediment by benthic organisms". As part of MOEE sediment quality-benthic macroinvertebrate community surveys in 1990, additional surficial sediment samples were collected at 10 stations in the upper St. Clair River near the Chemical Valley, and at 5 stations from the Algoma Slip in the upper St. Marys River. These were tested in laboratory sediment bioassays to assess the lethal and sublethal effects of sediment-associated contaminants to mayfly nymphs (*Hexagenia sp.*), midge larvae (*Chironomus tentans*) and to juvenile fathead minnows (*Pimephales promelas*). Contaminant bioaccumulation was also examined through analysis for chlorinated hydrocarbons of fish exposed to the St. Clair River samples. The St. Clair River bioassays showed differences in test organism sensitivity and also identified localized contaminant effects. Tissue concentrations in test fish were significantly correlated with related sediment concentrations of chlorinated benzenes. Bioassay results for the St. Marys River indicated that sediments from 4 of 5 stations were acutely toxic to all three test organisms. These toxic effects were associated with high concentration of PAHs and oils and greases. Bioassay results tended to correlate with observed alterations to benthic macroinvertebrate community structure. This assessment approach, combining sediment biassays with sediment quality and benthic community assessment, has proven beneficial in Stage 2 of the Remedial Action Plan process for these Areas of Concern.

CHEMICAL RELATIONSHIPS IN DETROIT RIVER FISH.

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ABSTRACT

Hydrophobic organic chemicals have been observed to accumulate in greater concentrations in the tissues of predators than in their prey. The concept of food-chain accumulation or biomagnification is proposed to explain the observed increase in tissue chemical concentration with trophic level in a natural fish community. The extent to which various chemicals biomagnify and the mechanism of biomagnification are at present, poorly understood. In this study benthic invertebrates and fish were collected from the Detroit River for analysis by electron capture gas chromatography for chlorinated benzenes and PCBs. Feeding relationships in this fish community were determined by detailed analyses of fish stomachs. The data indicate that chemical uptake via food-chain processes was most important for chemicals of high octanol/water partition coefficient (K_{ow}) and for fish at upper trophic levels in the community. GC-ECD analysis of stomach contents suggest a mechanism of biomagnification operating within the digestive systems of these fish.

INTRODUCTION

The biomagnification of hydrophobic organic chemicals in fish communities has been demonstrated in several studies (Oliver and Niimi 1998, Clark and Mackay 1991, Evans et al. 1991), however the mechanism of biomagnification is not well described (Gobas et al. 1993). The role of the chemical and physiological processes underlying a mechanism of biomagnification in effecting community level biomagnification is not well understood (Russell et al. 1995). This study quantifies the degree of biomagnification in a natural fish community and to relate the magnification of organochlorines from dietary uptake in individuals to community level biomagnification.

METHODS

Fish were collected from the Detroit River near Peche Island by seine, gill net, trap, or hook-and-line. Benthic invertebrates were collected by ponar or by hand from the same location. Benthic invertebrates collected included caddisfly larvae, unionid mussels, and crayfish. These organisms were assigned a trophic position of 1. Forage fish sampled included emerald shiner, spottail shiner, brook silversides, and gizzard shad. Forage fish were assigned a trophic position of 2. Predatory and benthic feeding fish collected included rock bass, freshwater drum, white perch, white bass, redhorse, yellow perch, and stonecat and were assigned a trophic position of 3. Trophic position assignments were based on diet analyses of the organisms collected. Samples (1-5 g) were prepared for electron capture gas chromatography by grinding by mortar and pestle in anhydrous sodium sulfate. Cleanup was done on activated florisil (60/100) mm mesh). Samples were analyzed for pentachlorobenzene (QCB), hexachlorobenzene (HCB), octachlorostyrene (OCS), p,p'-DDE, PCB #52, PCB #87, PCB #101, PCB #138, PCB #153, PCB #180. Chemical concentrations were expressed on a µg chemical per kg lipid basic. Chemicals were grouped by a principal component analysis and differences in chemical accumulation between the trophic positions were tested with a multivariate analysis of variance on component scores.

RESULTS AND DISCUSSION

Principal component analysis grouped the 10 chemicals into 3 components based on their behaviour in the environment. Component 1 explained 53.6% of the variance in the data set and included DDE and all PCBs. Component 2 explained 11.4% of the variance and included only QCB. Component 3 explained 24.2% of the variance and included HCB and OCS. The mean log K_{ow} of component 1, or the high K_{ow} component was 6.5. The log K_{ow} of component 2, or the low K_{ow} component was 5.0 and the mean log K_{ow} of component 3, or the mid K_{ow} component was 5.9. The MANOVA showed a highly significant difference in chemical accumulation between the 3 trophic positions for the high K_{ow} component, a marginally significant difference in chemical accumulation between trophic levels for the mid K_{ow} component, and no significant difference between trophic positions for the low K_{ow} component. Chemical concentrations in the tissues of the organisms studied increased with increasing trophic position for the high (log $K_{ow} = 6.5$) and mid (log $K_{ow} = 5.9$) K_{ow} chemicals. This demonstrated that biomagnification was occurring for these chemicals and that low K_{ow} (log $K_{ow} = 5.0$) chemicals did not biomagnify.

Diet analyses indicated that rock bass and stonecat were crayfish specialists at this site. Gas chromatography of the predator tissues, the prey tissues (crayfish), and predator intestinal contents (digested prey) showed increasing chemical concentrations from prey to predator intestinal contents to predator for both rock bass and stonecat for high and mid K_{ow} chemicals. A similar increase in chemical concentration was not seen for the low K_{ow} chemical. The pattern of biomagnification was observed to be the same for the individual dietary analyses as was observed for the community level analysis. This suggests that the mechanism of community level biomagnification may be a consequence of the intestinal physiology of individual fish.

CONCLUSION

Biomagnification was observed to be most important for fish occupying high trophic positions in the community and for high K_{ow} chemicals. Diet analyses indicated a mechanism of biomagnification operating within the digestive tract of individual fish. Chemical accumulation that was similar to the community level accumulation.

ACKNOWLEDGEMENT

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REFERENCES

Oliver, B.G., and A.J. Niimi. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ. Sci. Technol. 22: 388-397.

Clark, K.E., and D. Mackay. 1991. Dietary uptake and biomagnification of four chlorinated hydrocarbons by guppies. Environ. Toxicol. Chem. 10: 1205-1217.

Evans, M.S., G.E. Noguchi and C.P. Rice. 1991. The biomagnification of polychlorinated biphenyls, toxaphene and DDT compounds in a Lake Michigan offshore food web. Arch. Environ. Contam. Toxicol. 20: 87-93.

Gobas, F.A.P.C., J.R. McCorquodale and G.D. Haffner. 1993. Intestinal absorption and biomagnification of organochlorines. Environ. Toxicol. Chem. 12: 567-576.

Russell, R.W., R. Lazar and G.D. Haffner. 1995. Biomagnification in Lake Erie white bass (*Morone chrysops*). Environmental Toxicology and Chemistry. (In press).

TERATOGENIC AND GENOTOXIC RESPONSES OF LARVAL *Chironomus DIPTERA: CHIRONOMIDAE*) TO CONTAMINATED (DETROIT R.) SEDIMENT.

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Chironomids are acknowledged as important indicators of the effects of sediment- bound contaminants. Their ubiquity makes them suitable biomonitors for both large and small systems. Surveys of chironomids from 6 sites on the St. Clair/Detroit River system indicated that of 18 genera examined, only *Chironomus* and *Phaenopsectra* are both widespread and exhibit increased incidences of mouthpart deformities in contaminated habitats. However, no cause/effect relationship has yet been documented. In the laboratory, we exposed *Chironomus* larvae to mixtures of polluted, (Detroit River, MI) sediment diluted with uncontaminated, synthetic sediment (sand, sculptor's clay, potting soil). Groups of 12 2nd-instar *Chironomus* were grown to 4th instar (10 d) in water-filled 1-L jars containing 300 mL of Detroit River: synthetic sediment mixture ratios of 1:0, 1:1, 1:3, 1:7, 1:15 or 0:1. Surviving larvae were each examined for deformities of the mentum. Additionally, polytene chromosome preparations were made from salivary glands of the same animals. Giant chromosomes were examined for reduced relative size of the nuclear organizer (NOR), an indication reduction in general RNA synthesis activity. Incidence of chironomid deformities from control (0:1) sediments (±1 SE) was 7.9±1.6% (N=268): 4±1.5% of 178 control larvae examined displayed NOR reduction. Incidence of mentum deformities and of NOR reduction increased linearly with each doubling of contaminant concentration, from 16.8±2.1% (N-313) at 1:15 to 29.0±4.0% (N-261) at 1:0 for deformities, and from 5.8±2.1% (N=120) to 24.2±3.5% (N=149) for NOR reductions. NOR reduction in a larva was unrelated to mentum condition (X^2 test of independance p>0.60), indicating that these are independant responses to contaminant stress.

IN SITU FATHEAD MINNOW FECUNDITY AND OFFSPRING GROWTH ASSESSMENT TO DETERMINE THE LONGTERM BIOLOGICAL EFFECT OF INDUSTRIAL, MUNICIPAL AND RUN-OFF DISCHARGES INTO THE ST. CLAIR RIVER.

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ABSTRACT

To determine the ongoing impact of discharges to the St. Clair River an in-situ bioassay was instituted to determine the fecundity rates of fathead minnows (*Pimephales promelas*) as well as an assessment of the histopathological response of offspring. In 1992, testing of one generation was initiated. In 1993, testing included the monitoring of offspring (2nd generation) from the 1st generation as well as the continued monitoring of the 1st generation. The experimental design included a control station located upstream of all industrial and urban inputs and a test downstream station. Monitoring was conducted using a flow-through apparatus that allowed for monitoring of the river water quality from routine discharges and spill events. The fathead minnow testing results showed no station effect for the number of eggs or number of batches produced in either year or generation. The per cent egg survival was significantly higher at the downstream station in 1993 for both age classes only. There was no significant difference in survival of eggs when comparing 1st generation to 2nd generation fathead minnows in 1993. Histopathological examination of 2nd and 3rd generation offspring showed no apparent differences between locations. Monitoring results would suggest that St. Clair River water quality at the downstream location has no detrimental effect on fathead minnow fecundity and growth.

INTRODUCTION

Located along the Ontario side of the St. Clair River are a number of industrial facilities including petroleum refineries, petrochemical, organic, and inorganic plants and thermal electric generating facilities all of which discharge directly or indirectly to the St. Clair River. Other sources of discharges include several municipal wastewater treatment facilities, urban and agricultural non-point sources, and combined sewer overflows. Environmental studies have indicated a historically degraded environment characterized by poor water quality and sediment contamination that is progressively improving (Tarandus 1993, OMOE and MDNR 1991, LIS 1989). These improvements in the St. Clair River environment have been attributed to reduced contaminant loading to the river and natural recovery (OMOE and MDNR, 1991).

The premise of this study views a healthy ecosystem where species are capable of growth, reproduction, and survival. If an ecosystem has been altered creating undesirable conditions then one or more of these parameters can be affected. The design of this study successfully incorporates the parameters of growth, reproduction (fecundity), and survival as measures of ecosystem health. Fecundity has been indicated as the most sensitive indicator of toxicity (Sutter et al. 1987). The purpose of this study was to assess the quality of St. Clair River water by exposing various life stages of the fathead minnow to St. Clair River water upstream and downstream of the Sarnia/Lambton study area (Fig. 1). The study incorporates a flow-through design. The approach evaluates the biotic response to water conditions which exist during the entire monitoring period, on a continuous basis.

Biomonitoring stations are located at two sites on the St. Clair River. The upstream (control) site is located just south of the upper reach of the river and receives water from Lake Huron. Incoming water from the upstream location represents the background water quality before receiving any municipal, industrial and agricultural discharge waters from within the Sarnia/Lambton study area. The downstream site Is located south of Courtright. Incoming water from the downstream location contains the sum of all point source and non-point source discharges from the Sarnia-Lambton urban, rural and industrial areas to that point. Data from the two biomonitoring stations were compared to determine the net effect of river water quality on the various life stages of fathead minnows.

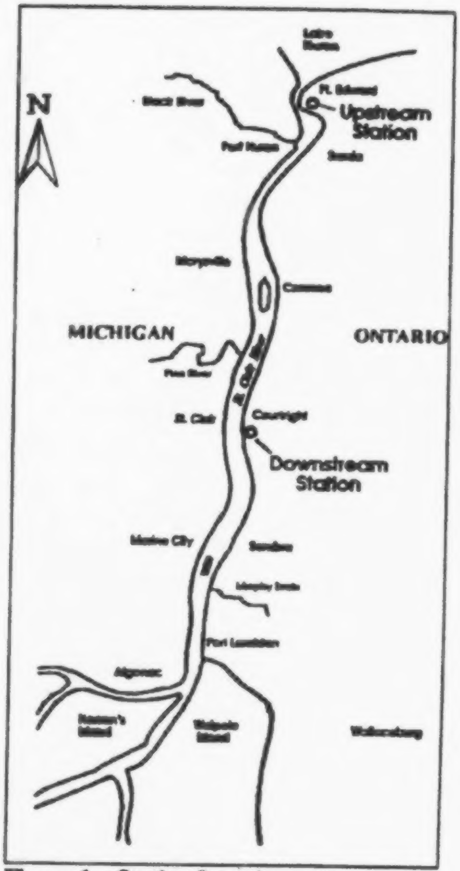

Figure 1 - Station Locations

The fathead minnow study was designed to determine life-cycle exposure effects on the reproductive success, growth, and survival of 1st and 2nd generation fathead minnows. The 1st generation being the fathead minnow breeding pairs first initiated in 1992 and the 2nd generation being the offspring of the 1st generation. Specifically, the objective of this program was to determine whether differences existed between the control and downstream locations as defined by the biological response of each generation. The responses or endpoints monitored included; the number of eggs produced, number of egg batches produced, rate of egg survival and adult survival. This evaluation thus provides an evaluation of one generation of fathead minnows for essentially their complete life-cycle. This being birth through to reproduction. In addition 2nd and 3rd (offspring of 2nd) generation fathead minnows, were sacrificed for histopathological assessment. This paper summarizes results of a fathead minnow growth and fecundity assessment first initiated in May 1992 and completed January 1994.

METHODS

This section describes in detail the fathead minnow culture techniques that were used in a flow-through experimental system located at the upstream and downstream locations on the St. Clair River. The practices employed in this study were based on methods recommended by Environment Canada (Environment Canada 1992) and the United States' Environmental Protection Agency (Denny 1987). All materials used in the construction of the culture facilities were considered inert and were tested for any adverse effects on the test organisms.

Fathead Minnow Culture: Each station location consisted of tanks of breeding adults, juvenile rearing tanks, and a hatching incubator for the eggs. The breeding and rearing tanks were 40 L glass aquaria with standpipe drains, to which a continuous flow of raw river water was delivered. At the upstream station a header tank was constructed to alleviate any possible problems from gas supersaturation of incoming water. No header tank was located at the downstream station as gas supersaturation of the water was not a problem because the water intake was located at a shallow depth compared to the upstream station. Original adult breeding sets (1st generation) were 10 months old at the beginning of the 1992 experiment, and originated from Pollutech's fathead minnow culture unit which was comprised of a mixed stock of Ontario Ministry of the Environment & Energy and Environmental Protection Agency (EPA) cultures. In November 1992, additional breeding tanks were installed at each of the stations to facilitate isolation and monitoring of 2nd generation fathead minnows.

Breeding Tanks: The spawning or breeding tanks were designed to simulate conditions in nature most conducive to spawning. Since the fathead minnows spawn in spring and summer in the wild, the culture systems were maintained to simulate the natural spawning conditions of daylight; a photoperiod of 16 hours light and 8 hours dark was maintained (Denny 1987). Fathead minnows have optimal reproduction at 25°C, however the temperature of the breeding tanks was dependent on the river water temperature. Breeding pairs in each tank were fed frozen brine shrimp once daily as a supplement to the incoming natural food source. At the beginning of the study, breeding pairs were watched closely to ensure all 10 pair at each station were viable. At the start of study in 1992 if a breeding pair was observed not to be breeding, one of the fish was replaced. If the adults died during the course of the study a new adult was introduced as quickly as possible. Extra 1st generation adult fathead minnows of the same age group were kept in spare tanks at each station. This pool of fathead minnows though not monitored for reproduction did represent individuals of a similar age and exposure, and were acclimated to test conditions. All 1st generation pairs were taken from the same age group and gene pool. Spawning substrates were 10-12 cm pieces of 4 inch diameter PVC pipe cut longitudinally in half. The semi-circular substrates were placed in the breeding tanks, cut edge down. This simulated the natural overhang conditions preferred by the fathead minnows.

Egg Incubation Units: Fathead minnows tend to spawn in the early morning. The spawning substrates were inspected daily for eggs. In nature the male protects, cleans and aerates the eggs until they hatch, but under culture conditions it is necessary to remove the eggs from the tanks to prevent the adults from eating some of the eggs. Once removed from the tanks, the substrates containing the eggs were placed into incubation vessels containing St. Clair River water for the station location. The incubation vessels were placed in a temperature controlled water bath and aerated during the incubation period. Eggs were incubated following Environment Canada (1992) procedures.

Data Collection: During the reproductive periods of each year, May through October, daily inspections and feeding of the breeding tanks were conducted. From November 1992 through May 1993, the winter period of low water temperatures and metabolic activity, inspections and feeding of breeding and grow-out tanks were restricted to every other day. When eggs were present the substrates were removed, subsequently replaced with another, and the number of eggs recorded. Daily inspections of eggs were made to determine egg mortality rate. Dead eggs, if present, were removed and recorded daily. The day of hatching was also recorded.

Histopathological Analysis: Forty fathead minnow grow-outs from the 2nd as well as the 3rd generation (20 from the upstream and 20 from the downstream locations) were sacrificed for histopathological examination at approximately 129 and 131 days of age, respectively. The fish were not fed for 24 hours prior to sacrificing in order to clear brine shrimp from their intestinal tracts. Each fish was assessed for gross external pathology (ie. gross deformities, eyes, gills, fins, etc.). Wet weight and total length of the 40 fathead minnows were also recorded. Weights (in grams) were determined to three decimal places using a Mettler H35AR balance. Lengths were recorded to the nearest millimetre.

For each sacrificed fish the tail was severed below the anal port, and the ventral surface was slit from the anal opening to the cardiac region using small scissors. Each specimen was then placed in Bouin's fixative for 24 hours. In this initial stage a small syringe was used to flush the abdominal cavity and to inject the solution into the mouth of the specimen. The specimens were then passed through two changes of 70% ethanol for 24 hours each. The specimens were then stored in a 4% Formaldehyde, 0.22M Hepes Buffer. Twenty fish (10 from each station) from each generation were sent for histopathological work. The other twenty remaining fish were archived. Histopathological work was performed by Experimental Pathology Laboratories (EPL) located in Henden, West Virginia with specimens submitted as groups 1 and 2 as to avoid bias.

Histopathological examinations of the fathead minnows included examination of the bone vertebra, brain, chromaffin tissue, corpuscle of stannius, esophagus, eye, gallbladder, gill, gonad, heart, haematopoietic tissue, interrenal tissue, intestine, kidney, liver, nares, pancreas, peripheral nerve, pineal organ, pituitary, pseudobranch, skeletal mussel, skin, spinal cord, spleen, stato-acoustic organ, swim bladder, thymus, thyroid tissue and urinary bladder.

Statistical Methods: The combined 1992-1993 fathead minnow data was analyzed using a variety of methods. The best approach would have been a multivariate analysis using number of eggs, number of batches and per cent survival as the responses. However, this would require the assumption of a joint multivariate normal distribution which is unlikely, given that none of the marginal distributions are normal. Thus a series of univariate tests were run, analyzing each response separately. Larval grow-out weights and lengths were analyzed using a Student's "T" test.

RESULTS

Data Presentation: In 1992 and 1993 the 1st generation started reproduction in June. The 2nd generation, not fully mature started reproduction by early August of 1993. Detailed data summaries for this study can be found in Pollutech (1993 and 1994). Table 1 provides an overall summary of the total number of eggs and batches produced for each generation and year.

Analysis of "Number of Eggs Data"

Table 2 summarizes the mean number of eggs per breeding tank per month. This summary for presentation purposes, only includes the specific breeding time period. A Wilcoxon Signed Ranks Test was used to analysis the data. Analysis of the data set combined over both years revealed no year effect. Subsequent analysis for each year and age group revealed no effect of station. The models fitted were: (a) number of eggs = station (year=1992, 1st generation); (b) number of eggs = station (year=1993, 1st generation); and (c) number of eggs = station (year=1993, 2nd generation). Thus there was no difference in the number of eggs produced between stations for either year or fathead minnow age class.

Table 1: Summary of the Total Number of Eggs and Batches Produced

Year	Generation	Total Number of Eggs		Total Number of Batches	
		Upstream	Downstream	Upstream	Downstream
1992	1st Generation	20680	21737	110	98
1993	1st Generation	15237	16807	89	92
	2nd Generation	11705	8633	62	57

Table 2 Summary of Mean Number of Eggs per Tank per month

Variable	Upstream	Downstream	Statistical Interpretation
1st Generation-1992	627 ± 399	639 ± 630	No Difference
1st Generation-1993	492 ± 402	622 ± 472	No Difference
2nd Generation-1993	616 ± 480	432 ± 327	No Difference

Analysis of "Number of Batches"

Table 3 summarizes the number of egg batches produced per breeding tank per month. This summary for presentation purposes, only includes the specific breeding time period. Univariate diagnostics for the response "number of batches", indicated that the data followed a Poisson distribution. The model

number of batches = year + station,

was fit as a generalized linear model (GLIM), using a Poisson response distribution and identity link.

The effect of year was not significant. The model was refit, omitting the variable year, and stratifying over year and age class as follows:

number of batches = station (year=1992, 1st generation)
number of batches = station (year=1993, 1st generation)
number of batches = station (year=1993, 2nd generation).

The effect of station was not significant in any analysis.

Table 3 - Summary of Mean Number of Batches per Tank per month

Variable	Upstream	Downstream	Statistical Interpretation
1st Generation-1992	2.2 ± 2.3	2.2 ± 2.0	No Difference
1st Generation-1993	1.8 ± 1.9	1.8 ± 2.3	No Difference
2nd Generation-1993	1.2 ± 1.9	1.1 ± 1.8	No Difference

Analysis of "Percent Survival": Table 4 summarizes the percent egg survival. This summary for presentation purposes, only includes the specific breeding time period. The response egg survival is a binomial response and consequently was analyzed using logistic repression. This was done under the GLIM family using a normal tolerance distribution and logit link. An overall model was fitted to assess the significance of the variable year, using the model

percent survival = year + station.

The effect of year was found to be significant. The data was stratified according to year and age and the following models were fit.

percent survival = station (year=1992, 1st generation)
percent survival = station (year=1993, 1st generation)
percent survival = station (year=1993, 2nd generation)

The effect of station was not significant in 1992 but significant in 1993 for both the 1st and 2nd generation fathead minnows. A comparison of the percent survival eggs between the 1st and 2nd generation in 1993 showed no significant difference, meaning the 2nd generation egg survival was no different than the 1st generation.

Table 4 Summary of Percent Egg Survival

Variable	Upstream % Survival	Downstream % Survival	Statistical Interpretation
1st Generation-1992	57.2 ± 28.8	66.1 ± 30.6	No Difference
1st Generation-1993	25.7 ± 26.4	50.6 ± 32.6	Significant Difference
2nd Generation-1993	32.9 ± 27.7	61.2 ± 26.5	Significant Difference

Adult Survival: From June to October of 1992 a total of five males and six females died at the upstream station. These mortalities all occurred at the beginning of the experiment and were attributed to gas bubble disease which was caused by gas supersaturation in the water. Gas supersaturation occurred because of the location of the intake pipe at the upstream station. The pipe inlet is at the bottom of the river, at a depth of approximately 10 to 12 meters. Water pumped from this depth is usually saturated with dissolved gases. As the water is pumped up to the surface, pressure is decreased and the water becomes supersaturated with the gases. Supersaturation of the water is normally recognized by the presence of gas bubbles on the walls of the tanks. Gas bubble disease is defined as a gas embolism in various organs and cavities of the fish, caused by supersaturation of gas in the blood (Piper et al. 1982). No mortalities occurred at the downstream station from June to October of 1992.

Incidental mortalities occurred in November, 1993 as a result of repairs to the water intake at the downstream station. From the middle of November, 1993 through January 10, 1994 a total of 3 males at the upstream station and 2 males and 1 female at the downstream station were found dead. No mortalities were observed in the 2nd generation breeding tanks during this time period.

Histopathological Analysis: In both submissions for the 2nd and 3rd generation offspring the pathological report indicated no apparent histological differences between the fathead minnows groups exposed to upstream and downstream St. Clair River Water. Any of the observations noted in the pathological reports were not considered to be life threatening. Detailed histopathological reports have been summarized in Pollutech (1993, 1994).

Larval Weight and Lengths: Table 5 and 6 summaries the grow-out weights and lengths, respectively, at the time of submission for histopathological examination.

Table 5 - Summary of Average Larval Grow-out Weight

Generation	Average Larval Weights (g)		
	Upstream	Downstream	Statistical Interpretation
2nd	0.330 ± 0.124	0.365 ± 0.174	No Difference
3rd	0.070 ± 0.017	0.081 ± 0.025	No Difference

Table 6 - Summary of Larval Grow-out Lengths

Generation	Average Larval Lengths (mm)		
	Upstream	Downstream	Statistical Interpretation
2nd	34.0 ± 3.4	33.9 ± 5.4	No Difference
3rd	20.9 ± 1.8	21.6 ± 2.2	No Difference

Statistical analysis of the 2nd generation 129 day and 3rd generation 131 day grow-outs showed no difference existed between weights and lengths between stations for each respective years. A noticeable difference between generations does exist.

DISCUSSION

In 1992 and 1993 no differences existed between the stations for either generation when comparing the number of eggs produced and number of egg batches. Though there was no difference in egg survival between stations in 1992, there was a detected difference in 1993 with egg survival being greater at the downstream station for both the 1st and 2nd generation results. This difference in survival may be attributable to increased nutrition available to the downstream fathead minnows. Downstream fathead minnows as well as upstream fathead minnows would have had their diets supplemented by incoming food. This nutritional component may be higher downstream because of potentially higher productivity of St. Clair River waters at the downstream station area. It is presumed this added nutritional component would add to the overall viability of the eggs to hatch. It is not known why this difference was not detected in 1992. Though there was a difference between stations for egg survival in 1993, there was no significant difference in survival of eggs produced by the two fathead minnow age classes in 1993 meaning 1st generation egg survival was comparable to 2nd generation egg survival.

There were no apparent histologic differences between 2nd generation (129 day grow-outs) and 3rd generation (131 day grow-outs) fathead minnows exposed to upstream and downstream water. Though some mild to moderate abnormalities did occur their frequency and occurrence were not considered to be life threatening or relatable to environmental factors. There was also no difference in weights and lengths between the upstream and downstream groups for each respective generation at the time stocks were sampled for histopathological examination. There was a marked reduction in size of the 3rd generation fathead minnows when compared to the relatively same age 2nd generation minnows. This is probably attributable to the colder seasonal period the 3rd generation fish where reared compared to the 2nd generation grow-out period.

The 3rd generation offspring could not be obtained until the later part of the breeding period (September, 1993). The 2nd generation fathead minnows did not attain sexual maturity until the early part of August, 1993; while the 1st generation breeding pairs, already sexually mature, initiated reproduction in June of 1992 and 1993.

Though specific occurrences of mortality occurred in the 1st generation breeding sets no environmental agent was attributed as the causative factor. Specific instances of mortalities at the beginning of this study and in November of 1992 were attributable to problems associated with the apparatus. Other occurrences of mortalities were considered sporadic and were considered attributable to natural attrition. No mortalities were observed in the 2nd generation breeding pairs.

From these results it may be concluded that differences in water quality, between the upstream and downstream stations had no significant detrimental effect on the fecundity, health, histopathological response and continued survivorship of the fathead minnows located at the downstream station.

From these results it may be concluded that any differences that may have occurred in water quality between the upstream and downstream stations had no detrimental effect on the fecundity, health, histological response and continued survivorship of the fathead minnows located at the downstream station. Utilizing these results as a general indicator of health this study would suggest that the biological water quality of the St. Clair River downstream of Sarnia's main urban and industrial areas is of a sufficient quality to maintain and sustain survival, growth and reproduction of aquatic biota.

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This study was commissioned by the Lambton Industrial Society (L.I.S.) a non-profit, industry-funded, environmental co-operative consisting of 15 member companies operating in the Sarnia/Lambton area. The LIS has monitored the environment in the Sarnia and surrounding area for the past 40 years through programs to assess the quality of ambient air, water, soil, and groundwater. The authors would also like to express our appreciation to Mr. Scott Munro, General Manager of the Lambton Industrial Society for his review of this transcript. The authors would also like to acknowledge the staff of Pollutech, Clay Ferguson and Kara Sparks whose dedication to the field efforts has been appreciated. The authors would also like to extend their appreciation to Barry Zajdlik, University of Waterloo for completing the statistical analysis.

REFERENCES

Denny, J.S. 1987. Guidelines for the Culture of Fathead Minnows *Pimephales promelas* for use in Toxicity Tests. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota. EPA/600/3-87/001.

Environment Canada. 1992. Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows. Conservation and Protection, Ottawa, Ontario. Report EPS 1/RM/22.

Lambton Industrial Society. 1989.

Ontario Ministry of the Environment and Ministry of Natural Resources. 1991.

Pollutech. 1993. Fathead Minnow Life-cycle Effects Monitoring - 1992. Final: October 15, 1993.

Pollutech. 1994. Fathead Minnow Life-cycle Effects Monitoring 1992 - 1993. Draft Report September 10, 1994.

Sutter, G.W. II, R.A.E. Rosen, E. Linder and D.F. Parkhurst. 1987. Endpoints for response of fish to chronic toxic exposures. Environ. Toxicol. Chem. 6: 793-809.

Tarandus. 1993. Analysis of the 1990 Benthic-invertebrate Samples From the St. Clair River. For the Ontario Ministry of the Environment Southwestern Region. Tarandus Associates Limited.

METAL TOXICOLOGY
SESSION CHAIR: R.P. LANNO

REASSESSING INTERACTIONS BETWEEN BIOENERGETICS AND CADMIUM BIOACCUMULATION IN RAINBOW TROUT. M.D. Dutton[1], B. Zajdlik[1], D.G. Dixon[1], and J.F. Klaverkamp[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1; [2]Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, MB, R3T 2N6.

Bioenergetics-based models are widely used to describe the bioaccumulation of Cd and other environmental contaminants in fish. The main justification for developing these models stems from the correlation observed between growth and contaminant concentration, which is generally thought to imply causality between bioenergetics and bioaccumulation. However, a correlation between two time-dependent phenomena does not necessarily indicate causality, and the bioenergetics interpretations to contaminant bioaccumulation may be questionable. This research was initiated to test the bioenergetics-based models of contaminant bioaccumulation in trout, using dietary Cd. We found that: (1) by increasing the ration of food containing a given Cd concentration, growth rate increased without an accompanying increase in hepatic bioaccumulation rate; (2) growth rate was inversely related to size, although Cd bioaccumulation rate was size-independent. These findings did not corroborate the assumptions of the bioenergetics approach, and support the view that growth (ie. bioenergetics) and bioaccumulation are correlated, but independent phenomena. Since Cd and energy-containing molecules (eg. sugars, protein, lipid) are taken up from the gut via transport-specific mechanisms, it should be possible to alter Cd accumulation independent of growth. In our experiments, the absence of a ration-specific response in Cd accumulation rate probably relates to such an interpretation. For any contaminants which are taken up independent of energy-containing molecules, it is conceivable that bioaccumulation could be successfully modelled in a framework simpler than that of the bioenergetics-based approach.

SPACIAL AND TEMPORAL VARIABILITY IN METAL BIOAVAILABILITY AND TOXICITY OF SEDIMENT FROM HAMILTON HARBOUR, LAKE ONTARIO. G. Krantzberg. Ontario Ministry of Environment and Energy, 40 St. Clair Ave. W., Toronto, ON, M4V 1M2.

Trace metals in sediment from nearshore urban and industrialized centres of the Great Lakes are frequently at concentrations well above geological background values. Total metal content in sediment, however, is a weak predictor of sediment toxicity. This study examined the bioavailability of metals from Hamilton Harbour in Lake Ontario, and considered variability in metal forms on a temporal basis. Sediment from regions within Hamilton Harbour is highly contaminated with metals, nevertheless, not all metal contaminated sites were toxic to test organisms. Most sediment did elicit sublethal and/or lethal responses in bioassay organisms. Metal bioavailability, as measured by weak acid extractions, metal bioaccumulation by fathead minnows and sediment toxicity was greater in sediment collected in the fall as compared to sediment collected in the spring. Results of analyses of tissue residues in test organisms and the reduced toxicity observed in

sediment collected from some stations in the spring as compared to the fall, implicate trace metals and sediment oxygen demand as contributing to sediment toxicity. The suitability for colonization by benthic invertebrates of sediment in some areas of Hamilton Harbour appears to be limited by both contaminants and high sediment oxygen demand. Improving the oxygen regime of the harbour should result in improvements in the benthic invertebrate community directly, by providing a suitable oxygen regime for organisms less tolerant of temporal anoxia, and indirectly by decreasing metal bioavailability.

EFFET DU CHLORURE DE TRIBUTYLETAIN SUR LA CROISSANCE DE LA DIATOMEE MARINE: *Phaeodactylum tricornutum* (Bohlin). K. Mouhri[1], P. Marsot[2], E. Pelletier[2] et R. Saint-Louis[2]. [1]Départment de Biologie, Faculté des sciences semlalia, B.P S 15, Marrakech, Maroc; [2]Institut National de la Recherche Scientifique, Rimouski, QC, G5L 3A1.

Le Tributylétain (TBT) est considéré comme un composé organométallique toxique. Son utilisation comme ingrédient actif dans les peintures antisalissures pour les coques des bateaux peut occasionner une pollution locale des environnements côtiers. La toxicité des organoétains envers les organismes marins varie d'une espéce à l'autre et dépend également des caractéristiques physico-chimiques de l'environnement aquatique contaminé (Alzieu 1989). L'impact du TBT sur le phytoplancton marin a été étudié essentiellement sur la croissance, la productivité et la photosynthése (Beaumont et Newman 1986, Liying et al. 1990). Il est également établi que plusieurs espéces de microalgues accumulent le TBT et le dégradent en dibutylétain (DBT) et en monobutylétain (MBT) (Lee et al. 1989, Saint-Louise et al. 1994). L'objectif de ce travail est d'évaluer l'effet du chlorure de tribuylétain sur la croissance et le comportment d'une diatomée marine dominante de l'estuaire du Saint-Laurent: *Phaeodactylum tricornutum.*

Une culture confinée axénique de 20 litres de *P. tricornutum* (Eau de mer enrichie, Photopériode 16:24 Heures, 306 µEm-2s-1, T = 16°C) a été ensuite exposées durant une semaine à 0; 0,5; 2; 10 et 20 µg de TBT. Le dénombrement des cellules a été réalisé à l'aide d'un hématimètre de Neubauer. Le volume cellulaire moyen a été estimé avec coulter counter (sonde de 70 µm d'ouverture). Les sels nutritifs ont été mesurés à l'aide du Technicon Autoanalyser II. L'extraction des organoétains et leur analyse ont été réalisées selon le protocole de Reader et Pelletier (1992).

Nos résultats ont montré que l'inhibition de la croissance de l'algue étudiée augmente avec le degré de contamination du millieu de culture. La croissance de *P. tricornutum* n'est pas affectée par la plus faible concentration de TBT utilisée (0, 5 µg/L). Cette algue serait plus tolérante à ce niveau de contamination que d'autres espéces telles *Skeletonema costatum et Pavlova lutheri* (Beaumont et Newman 1986). L'impact le plus important a été observé avec des concentrations plus élevées de TBT (10 et 20 µg/L), la densité cellulaire a été réduite de 40 à 80% respectivement avec une augmentation du taux de mortalité des cellules algales qui atteint 20 à 40% de la densité totale.

L'analyse du volume cellulaire moyen dans les différentes cultures contaminées montre une nette augmentation du volume des particules avec le niveau de contamination. Le volume maximum a étéattein à 20 µg/L de TBT aprés 24 heures traduisant une agglomération des cellules de cellules de *P. tricornurum* sous l'effet de fortes concentrations du polluant. Ces cellules agglomérées sédimentent plus rapidement que les cellules non contaminées et peuvent constituer, dans le milieu naturel, une menace en raison de leur contenu non négligeable en butylétains sous sa forme la plus toxique (Wong et al. 1982).

Par ailleurs, nous avons constaté que la vitesse d'absorption des nitrates diminue signicativement avec le taux de contamination du milieu de culture. Le taux d'inhibition maximum a été obtenu à 20 µg/L et aprés 168 heures d'exposition au polluant.

L'analyse des organoétains a mis en évidence une accumulation rapide du TBT par les cellules dés les premiéres 24 heures. Cette accumulation augmente avec le degré de contamination pour atteindre une charge cellulaire maximale (10 µg/g de poids sec) à 20 µg/L et aprés une exposition de 72 heures au polluant. L'accumulation est accompagne accompagnée d'un processus de biodégradation du TBT en DBT souvent considéré comme produit moins toxique (Wong et al. 1982). Les concentrations de DBT dans les cellules algales augmentent avec la concentration du contaminant dans le milieu de culture. Cependant le rapport TBT/DBT augmente lorsque la concentration initiale de TBT est plus importante.

Ceci suggère que la capacité de dégradation du TBT par
P. tricornutum est affectée par le niveau de contamination du milieu où elle évolue.

En conclusion, nos observations suggèrent deux types d'impacts sur le milieu marin résultant des effets du TBT sur la diatomée étudiée:
i-l'un écologique, par la diminution de la biomasse et de la productivité algale (inhibition de la croissance, sédimentation acrrue);
ii-l'autre écotoxicologique sur le milieu benthique, par l'apport accéléré du TBT associé aux agrégats d'algues contaminées.

RÉFÉRENCES

Alzieu, C. 1989. L'étain et les organoétains en milieu marin: biogéochimie et écotoxicologie. Rapport scientifique et téchnique.

Beaumont, A.R., et P.B. Newman. 1986. Low levels of tributyltin reduce growth of marine microalgae. Mar. Poll. Bull. 17: 457-461.

Lee, R.F., A.O. Valkins et P.F. Seligman. 1989. Importance of microalgae in the biodegradation of tributyltin in estuarine waters. Environ. Sci. Technol. 23: 1515-1518.

Liying, Z., L. Xiankum et S. Bingyi. 1994. Toxic effects of organotin on marine Diatoms. Journal of Ocean University of Qingdao. 20(4): 125-131.

Reader, S., et E. Pelletier. 1992. Identification and determination of butyltin compounds by gas chromatography-ion trap spectrometry. Anal. Chim. Acta. 262: 307-314.

Saint-Louis, R. E. Pelletier, P. Marsot et R. Fournier. 1994. Distribution et effets du chlorure de tributylétain et de ses produits de dégradation sur la croissance de l'algue marine Pavlova lutheri en culture continue. Water Res. (sous presse).

Wong, P.T.S., Y.K. Chau, O. Kramar et G.A. Bengert. 1982. Structure Toxi of tin compounds on algae. Can J. Fish. Aquat. Sci. 39: 483-488.

COMPARAISON ENTRE L'UTILISATION D'UNE MOUSSE AQUATIQUE ET D'UN MOLLUSQUE À TITRE D'INDICATEUR BIOLOGIQUE DE LA POLLUTION MÉTALLIQUE. L. Pazdernik. Département de Chimie-Biologie, Université du Québec à Trois-Rivières, Trois-Rivières, QC, G9A 5H7.

Au Québec, le suivi de la qualité des cours d'eau représente un défi de taille dus au vaste bassin hydrographique et à la présence de substances toxiques à l'état de traces dans les eaux de surface. Les cours d'eau reçoivent des polluants de toutes sortes(organiques et inorganiques) menaçant les écosystèmes et pouvant nuire à la santé de l'homme. Pour l'instant les efforts sont surtout centrés sur l'utilisation d'indicateurs biologiques capables d'informer sur les polluants métalliques. Les avantages reliés aux traceurs sont la facilité d'utilisation, une bonne capacité à concentrer les métaux et des coûts minimes associés à leurs utilisations. Toutefois, lors d'études comparatives des biomoniteurs il faut tenir compte de la physiologie différente de ceux-ci. Les mousses accumulent et éliminent grâce à des échanges cationiques au niveau des parois cellulaires alors que chez les mollusques l'accumulation se fait dans les cellules en contact avec le milieu ambiant et il y a distribution dans les organes. Puisque les mousses accumulent plus vite que les moules, on considère qu'elles sont très utiles lors d'accidents écologiques alors que les moules peuvent facilement être utilisées pour le suivi d'une pollution chronique étant donné leur capacité d'accumulation plus lente. Jusqu'à présent, les mousses aquatiques n'ont pas fait l'objet de beaucoup d'études chez nous, mais il serait souhaitable qu'une attention particulière soit portée aux bryophytes pour l'évaluation environnementale. Or, considérant tous ces facteurs, et puisque les mousses réagissent plus vite aux agressions, nous pensons qu'il serait souhaitable dans le futur, d'utiliser ces organismes en parallèle, de manière à mieux comprendre les mécanismes impliqués dans la contamination.

COPPER KINETICS IN *HYALELLA AZTECA*: IMPLICATIONS FOR PREDICTING CHRONIC COPPER TOXICITY. U. Borgmann and W.P. Norwood. Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington, ON, L7R 4A6.

Metal accumulated by aquatic organisms provides a potentially far more useful indicator of toxicity than metal in water or sediment because only the bioavailable form of the metal is accumulated. For non-regulated metals, such as cadmium, higher concentrations in the body can be assumed to imply a higher risk of toxic effects. Essential metals such as copper and zinc, however, are strongly accumulated, even in pristine environments, and regulated by many species. A thorough understanding of metal uptake kinetics is, therefore, required before toxic effects can be inferred from accumulated metal. In *Hyalella azteca*, whole body copper concentrations are independent of body size for both control and metal exposed amphipods. Initial uptake rates are rapid, but copper concentrations during continuous exposure peak after about 1 week and then gradually decline back towards control levels. Chronic toxicity cannot, therefore, be inferred from wild or continuously exposed amphipods. Copper availability must be assessed using naive animals. Depuration rates for copper are much slower than the apparent depuration rates during uptake assuming a simple diffusion model, apparently because copper uptake displays saturation kinetics. The half-life of copper cannot, therefore, be estimated from

the uptake curve. Chronic mortality was significant at copper exposures which resulted in predicted copper accumulations of 1.8 µmole/g dry weight above background in previously unexposed amphipods after 1 week of uptake.

MODELLING METAL INTERACTIONS AT FISH GILLS: SILVER AND NICKEL.

R. Playle, N. Janes, L. Hollis and D. Horn. Department of Biology, Wilfrid Laurier University, Waterloo, ON, N2L 3C5.

Our approach is to experimentally determine metal-gill equilibrium binding constants and the number of metal-gill binding sites. These values are then inserted into an aquatic chemistry program (MINEQL$^+$) to predict metal-gill interactions and therefore toxicity to fish. Rainbow trout (*Oncorhynchus mykiss*, 1-3 g) were exposed to 0.09 µM Ag (10 µg/L) in synthetic soft water, in the presence or absence of complexing agents such as thiosulphate ($S_2O_3^{2-}$), DOC, and Cl^-, or the addition of competing ions such as Ca^{2+}, Na^+, and H^+. Fish gills were assayed for Ag using graphite furnace AAS. The binding constant for Ag-gill interactions was log $K_{Ag\text{-}gill}$=10.0 and the number of binding sites for Ag was 10 nmoles/g wet gill tissue. Silver complexation by DOC had an equilibrium constant of log $K_{Ag\text{-}DOC}$=9.0. An example of competitiion for Ag binding sites is Na^+, with log $K_{Na\text{-}gill}$=4.7. These and other experimentally-determined binding constants were used to successfully predict Ag accumulation on gills of rainbow trout held in a series of test waters. In contrast, Ni concentrations in gills are naturally high, so measuring Ni accumulation on gills at environmentally realistic Ni exposures is impractical by graphite furnace AAS. Instead of measuring Ni on gills directly, Na^+ loss from the fish to the test water was used as an index of Ni binding to gills. In this manner the influence of temperature on Ni-gill interactions was determined, and an estimate of the Ni-gill binding constant was made using EDTA (log $K_{Ni\text{-}EDTA}$=20.3) as a competitive ligand.

ESTIMATING FREE CU ION CONCENTRATION AT LOW LEVELS IN LAKE WATER.

P.G. Welsh[1], D.G. Dixon[1] and G.M. Mierle[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1; [2]Ontario Ministry of Environment and Energy, Dorset Research Centre, Dorset, ON, P0A 1E0.

According to the free ion activity model, divalent metal ions are acutely toxic and ligand bound metals are non-toxic. The usefulness of this model for chronic metal exposures is unclear, since the role of ligand bound metal complexes in chronic exposures is not well understood. In order to determine if chronic toxicity is a function of the free Cu ion (Cu^{2+}) or a combination of free and ligand bound Cu, the various Cu species must be measured. In soft water lakes, Cu is toxic to larval fish at very low total concentrations (5-20 µg/L) and measuring Cu speciation at these levels is difficult. Using a Cu Ion Specific Electrode (Cu-ISE) and equilibrium dialysis, we have attempted to determine Cu^{2+} concentration in soft water at realistic Cu concentrations. Both approaches have limitations. The Cu-ISE appears to respond to interfering substances which prevents determinations of Cu^{2+} at low total Cu concentrations (<0.1 µg/L). We were unable to obtain an estimate of Cu^{2+} by dialysis due to either equilibrium conditions not being met or infiltration of organic substances into the dialysate. Comparisons between chronic Cu toxicity, total Cu and operationally defined Cu^{2+} will be made.

ARE DECLINING METAL LEVELS RESPONSIBLE FOR THE RECOVERY OF DAPHNIA GALEATA MENDOTAE IN ACIDIC SUDBURY LAKES AFTER LIMING?

N.D. Yan[1], P.G. Welsh[2] and H. Lin[3]. [1]Ontario Ministry of Environment and Energy, Dorset Research Centre, Dorset, ON, P0A 1E0; [2]University of Waterloo, Waterloo, ON, N2L 3G1; [3]Shanghai Research Institute of Environmental Protection, Shanghai, PR China.

Daphnia galeata mendotae(DGM), one of the most common and abundant zooplankton species in Ontario, has recently re-appeared in Hannah and Middle Lake, two lakes historically stressed by acid and metals. These lakes were limed in the early 1970s. Since then, the lakes have remained non-acidic and metal levels have declined monotonically. DGM assumed dominance in Hannah Lake approximately 5 years before it did in Middle Lake. One hypothesis for this temporal displacement is that metal availability regulated recovery in the two lakes. We examined the chronic toxicity of Cu, Cd and Ni mixtures to DGM in the laboratory with Middle and Hannah lake water modified to reflect metal concentrations in 1976, 1981, 1985, 1989 and 1993. Cultures of DGM were established from both lakes. Survivorship and production of live neonates were sensitive indicators of past metal conditions. Lake waters were acutely lethal to both DGM populations immediately after liming (1976). The Hannah Lake DGM survived better and produced more offspring than Middle Lake DGM for all remaining simulated lake conditions. These results correspond well with field assessment of the timing of recovery of DGM in the two lakes.

MODELLING/POPULATION MODELLING
SESSION CHAIR: M. POWER

THE ATMOSPHERIC PATHWAY FOR PESTICIDE DEPOSITION TO THE GREAT LAKES. M.T. Scholtz[1], E.C. Voldner[2], C. Slama[1] and A. Li[2]. [1]ORTECH, Mississauga, ON, L5K 1B3; [2]Atmospheric Environment Service, Environment Canada, Downsview, ON, M3H 5T4.

The atmospheric transport of pesticides over regional and even global scales from the areas where they are applied is emerging as an important route for their deposition to water bodies. The emission of agriculturally applied pesticides from soil and vegetation and the meteorological influences on these emissions will be discussed. The partitioning of the pesticides between particulate and gas phases will be described and the relative importance of this partitioning to the deposition process will be assessed. Episodic emissions of pesticides due to their seasonal application, as well as the seasonal tilling of soils containing pesticide residues, could explain observed periods of high atmospheric loading which result in substantial deposition to regions which are remote from pesticide applications.

AN INTERPRETATIVE FRAMEWORK FOR MONITORING FISH POPULATION RESPONSES TO INDUSTRIAL DISCHARGE. W.N. Gibbons[1] and K.R. Munkittrick[2]. [1]Gibbons Environmental Research, 79A Oxford Street, Guelph, ON, N1H 2M5; [2] GLLFAS, Department of Fisheries and Oceans, Burlington, ON, L7R 4A6.

Canadian pulp and paper mills regulated under the amended Pulp and Paper Effluent Regulations (Federal Fisheries Act) are required to conduct an Environmental Effects Monitoring program at effluent receiving waters. An adult fish survey has been included in the program to identify possible changes in whole organism characteristics (growth, reproduction, energy storage, age) of fish exposed to pulp mill effluent. A monitoring framework was developed to facilitate the interpretation of survey data. Based on information describing age structure (mean age), energy expenditure (growth, reproduction) and energy storage (condition, liver size, lipid levels) of fish, several generalized response patterns have been identified from the published literature. The patterns represent characteristic responses to changes in food availability, adult mortality, recruitment failure and metabolic disruption. By comparing the observed fish characteristics of the fish survey with these patterns, the identity of the observed fish response can be determined, as well as the probable mode of action responsible for the change. The framework also provides a mechanism to focus and design appropriate follow-up studies.

CONSIDERING NATURAL GRADIENTS AND VARIABILITIES IN FISH POPULATION DYNAMICS. M.M. Gagnon[1], P.V. Hodson[2] and J.J. Dodson[3]. [1]Ecological Services for Planning Ltd, Guelph, ON, N1G 3M5; [2]Canada Center for Inland Waters, Burlington, ON, L7R 4A6; [3]Université Laval, Sainte-Foy, QC, G1K 7P4.

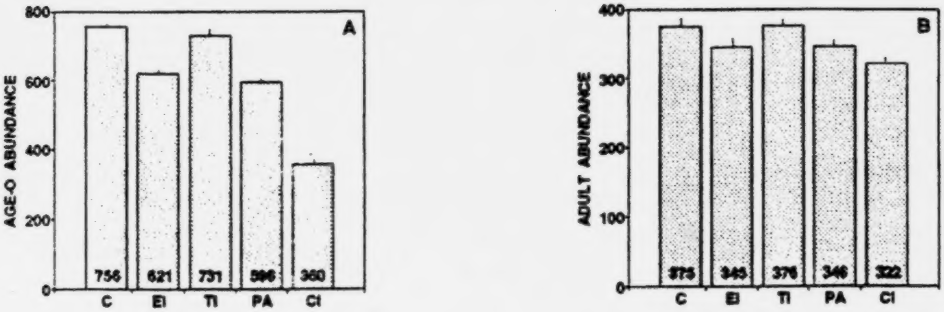
Responses to bleached kraft mill effluent (BKME) were measured in three populations of white suckers (*Catostomus commersoni*) in the St. Maurice River, Québec, and compared to the responses in three white sucker populations in a reference river. It has been hypothesized that exposure to contaminants would influence fish population dynamics by reducing age at maturity, modifying age distribution, and affecting fish condition and growth. However, BKME-exposed suckers showed a similar age at maturity and age distribution, and an increased lipid accumulation and growth relative to an upstream reference site. Suckers from the reference river also showed increased lipid accumulation and growth downstream. Natural gradients seem to camouflage the effects of contaminant exposure on some fish population parameters. Therefore, local environmental conditions should be considered when comparing dynamics of fish populations.

A MODELLING FRAMEWORK FOR ANALYZING ANTHROPOGENIC STRESSES ON BROOK TROUT POPULATIONS. M. Power[1] and G. Power[2]. [1]Department of Agricultural Economics, University of Manitoba, Winnipeg, MB, R3T 2N2; [2]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

Modelling provides a convenient and useful means of assessing the possible risks contaminant stresses pose for many populations. The use of models for predicting the impacts of human activity on populations, however, does not guarantee either their accuracy or utility. To be truly useful models must be properly constructed and validated. The literature has stipulated a number of necessary characteristics that population-level models designed to assess the effects of anthropogenic stresses should possess. These include: the production of data outputs consistent with the endpoints of regulatory interest; the ability to use available toxicological information; the ability to use available, or obtainable, population-level data; and the recognition of the importance of variability among individuals within a population. We used an individuals-based model validated against field data (Power and Power, in press) designed specifically for the population-level analysis of brook trout (*Salvelinus fontinalis*) that incorporated available biological life-history data and could be adapted to include consideration of anthropogenic stress e.g. exploitation and toxaphene exposure.

Populations respond to the aggregate of both anthropogenic and natural influences and, as a result, decisions about the fate and effects of chemicals based on laboratory experiments, or extrapolations of laboratory data, will be low in realism because they tend to ignore cumulative impacts and other aggregate events. Nearly any activity involves more than one kind of impact and more than one kind of adverse effect. Stresses as diverse as the physical degradation of habitat and chemical contamination will interact with other temporally and spatially coincident stresses to affect environmental integrity and measured endpoints in time and space. This implies that population studies should avoid undue reductionism and give explicit consideration to the multiple stresses, subsidies and interactions which can occur in complex environments.

The brook trout modelling framework was used to characterize the nature of exploitation and toxaphene stresses acting simultaneously on a population in terms of age 0 and adult abundance, survivorship and population size-structure. A no-stress control case was estimated against which the exploitation-only, toxaphene-only or the cumulative exploitation and toxaphene stress cases were compared to determine the extent and significance of impacts. The single stress case results were further used to predict the cumulative impacts by assuming additivity and the predictions compared to the modelled combined stress case results.



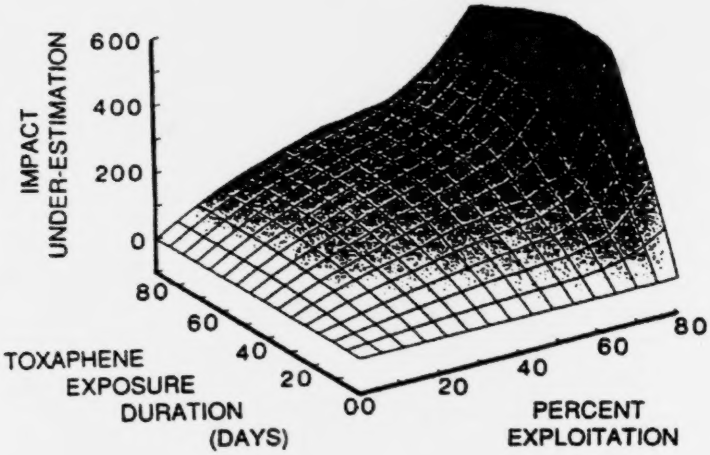
Panel A, above, compares age 0 abundance in a representative 0.25 ha area under the control [C], 60% exploitation stress [EI], 90 day toxaphene exposure stress [TI] and the cumulative stress [CI] cases. Also reported are the predicted abundance results [PA] derived using the additivity assumption. Actual abundance figures are reported at the base of each bar and vertical lines at the top indicate the size of the 95% confidence interval half-length. Panel B compares adult abundance for each of the same stress cases. The comparisons clearly indicate the inadequacy of the additivity assumption as a means of predicting cumulative impacts.

As a result of the initial comparison above, a designed experiment was completed using 9 levels of the exploitation, $\mathbf{e}$, and 7 levels for the toxicant exposure duration, $\mathbf{t}$, to examine the nature of the interaction between the stresses. The exploitation levels used were: 0 to 80%, in 10% increments. The design did not include values for $\mathbf{e}$ above 80% because values of $\mathbf{e} > 80\%$ resulted in local extinction. The duration of toxaphene exposure values used ranged from 0 to 90 days in 15 day increments. The data were then used to estimate the following models:

$$\begin{aligned} \text{Age 0} &= 952.17 - 118.13\mathbf{e} - 1.30\mathbf{t} - 2.62\mathbf{et} \qquad r^2 = 0.866 \\ \text{Adult} &= 389.24 - 149.34\mathbf{e} - 0.71\mathbf{t} - 2.62\mathbf{et} \qquad r^2 = 0.526 \end{aligned}$$

All p-values < 0.001. Prediction of the effects of varying either $\mathbf{e}$ or $\mathbf{t}$ can only be accurately made if the interaction between the two is included in the prediction. The population implications for the independent assessment of environmental perturbations (stresses) is clear. Routine assessment of the probable impact of a stress cannot be carried out without first determining whether the considered stress acts independently of all other concurrently acting spatial or temporal stresses. Given the pervasiveness of exploitation as a stress, the potential incremental effects of sublethal stresses on fish populations should not be dismissed on the weight of laboratory toxicity tests alone as laboratory tests are not designed to consider interaction with other stresses.

The relative importance of the main and interactive terms in determining abundance also suggests that the additivity model will lead to substantial under-estimates of the effects of cumulative impacts on a population. The figure below plots the differences between the modeled cumulative stress impacts and the predicted impacts, using the additivity assumption in terms of abundance losses versus the control case.



Under-estimate of Impacts Resulting from the Omission of the Interactive Term

The differences are calculated as modeled minus predicted additive impacts such that the resulting plotted values measure the degree to which the additivity assumption under-predicts. At low levels of either stress the differences between the two approaches are minimal. The differences between the approaches are marginal through the full range of either considered stress as long as the other stress remain low. This suggests that without adequate data sets field-based monitoring programs may not be able to detect significant differences between the two approaches. However, as both stress levels rise the differences between the two approaches also rise, eventually reaching a point of exponential divergence.

REFERENCE

M. Power and G. Power. 1994. A modelling framework for analyzing anthropogenic stresses on brook trout (*Salvelinus fontinalis*) populations. Ecological Modelling. (In press).

TOXICOLOGICAL STATISTICS
SESSION CHAIR: B.A. ZAJDLIK

ESTIMATION OF NO-EFFECT CONCENTRATIONS OF EFFLUENTS BASED ON LOG-LOGISTICALLY DISTRIBUTED NOEC TOXICITY DATA. H. Sloterdijk. Centre Saint Laurent, Environment Canada, 105 McGill, Montreal, QC, H2Y 2E7.

ABSTRACT

A procedure for the estimation of a "safe" concentration, using NOEC toxicity data, is presented. It is a statistical approach based on the theory that toxic responses (NOEC's, LC50's, etc.) are log-logistically distributed. A computer algorithm (called ETX) allows the extrapolation of percentiles with a certain confidence from a small set of toxicity data. The ETX algorithm, in essence, calculates the 5th percentile of the hypothetical logistic distribution from which the data are thought to derive (laboratory toxicity data). This 5th percentile can be considered a NOEC value, above which 95 % of the species seem relatively safe. This algorithm was applied to toxicity data on effluents of three industries on the St. Lawrence River. The data include results from Microtox, algal, and ceriodaphnia tests, and from the SOS-Chromotest (genotoxicity). The extrapolated NOEC values, expressed as toxic units (TU), can be used to rank the effluents, and to calculate "mass loadings" of TU's. Based on the effluent and the receiving waters flowrates, dilution ratios can be calculated, which may indicate sufficient or insufficient mixing or dilution.

INTRODUCTION

Ecotoxicological risk analysis has become a subject of great interest in environmental management. However, predicting how an ecosystem will respond to the stress of toxicants is a difficult task (Smith and Cairns 1993). Nevertheless, the objective of laboratory biotesting is to assess the potential impact of toxic chemicals on natural ecosystems and to extrapolate the risk factors. Simple risk analyses can be performed by the extrapolation of chronic toxicity data for various test species, using distribution models (Stephan et al. 1985, Kooijman 1987, Van Straalen and Denneman 1989, Wagner and Lokke 1991, OECD 1992, Aldenberg and Slob 1993). These authors assume that LC50 or NOEC values (No Observed Effect Level) density distributions for single test species and for all species in a community are either log-normal, log-triangular or log-logistical.

Van Straalen and Denneman (1989) developed a statistical model, based on Kooijman's (1987) approach (log-logistic distribution), which estimates the hazardous concentration (HCp) to p % of the species. This means that the probability of selecting a species with a NOEC smaller than HCp is p%. Since the true statistical parameters of the distribution curve are not known, computer simulated correction factors were introduced in the model. The extrapolation model of Van Straalen and Denneman, however, overpredicts the HCp more than 50% of the time. Aldenberg and Slob (1993), therefore, developed a modification of Van Straalen and Denneman's model, where the correction factors were construed for small sample size, i.e. a limited number (3 to 8) laboratory toxicity tests, allowing only a certain, pre-specified amount of overprediction. A computer algorithm (called the ETX model) was developed to carry out the calculation of the 5th percentile (HC5), using the statistical extrapolation techniques and correction factors for small sample size (Aldenberg 1993).

Outline of the Extrapolation Method:

The ETX algorithm calculates the 5th percentile of a hypothethical log-logistical distribution from which the toxicity data (usually NOEC's) are thought to derive. It is fairly similar to a log-normal distribution (Fig. 1), but has some mathematical features facilitating the extrapolation procedure. This 5th percentile is called the Hazard Concentration or HC5. The probability density distribution of its estimate is shown at the left in Fig. 1.

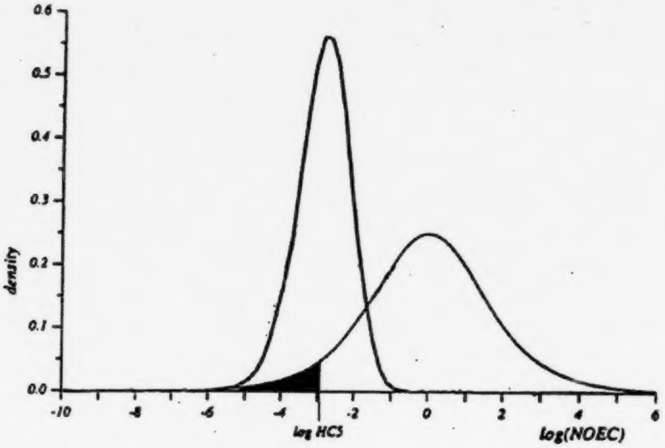


Fig.1. The standard logistic distribution of log(NOEC) values. Log HC_5 = -2.94 is the log Hazardous Concentration to be estimated; the distribution of the estimate itself is shown at the left. The fraction of the species harmed is shown shaded (from Aldenberg & Slob, 1993; with permission).

The midpoint value, the median or the 50th percentile and the left confidence limit or the 95th percentile of the HC5 distribution can be calculated or "extrapolated" from a small set of toxicity data with a certain confidence. The Goodness-of-Fit of the data is determined by a non-parametric test, the Kolmorogov-Smirnov D*sqrt(n) test, which is especially suitable for small-sized samples (Sokal and Rohlf 1981).

Aldenberg and Slob (1993) can be consulted for a more detailed treatise of the statistics behind the extrapolation method. It suffices to mention that if the true mean and standard deviation (μ and σ) were known, this HC5 can be easily calculated for the logistic distibution as:

$$\log HC_5 = \mu - 1.62 \cdot \sigma$$

The parameters μ and σ can be related to the location and scale parameter, $\alpha = 0$ and $\beta = 1$, respectively, such that $\mu = \alpha$, and $\sigma = (\pi/\sqrt{3}) \cdot \beta$. The location of HC5 is $(\mu - 1.62 \cdot 1.81) = -2.94$ (Fig. 1). One can estimate these parameters by the sample mean X and the sample standard deviation s. Thus,

$$\log HC_5 = X - K \cdot s$$

where K, the extrapolation constant, is 1.62 if we had complete information about the distribution curve. Aldenberg and Slob (1993) have generated, through Monte Carlo simulation, K values for different sample sizes (Table 1) in such a way that a prespecified confidence level (50 and 95%) was obtained, with a corresponding frequency of overprediction (50% and 5%, respectively; Fig. 2).

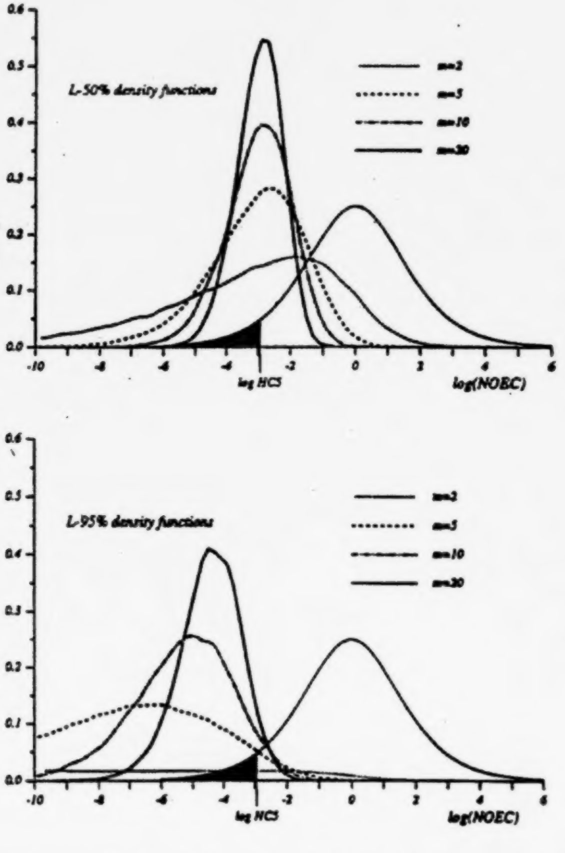


Fig. 2. Simulated variation of answers that result from using the 50 and the 95% confidence level for four different sample sizes (m), using the standard logistic density function $HC_5 = X - K \cdot s$, with K from table 1 (from Aldenberg & Slob, 1993; with permission).

Although, statistically, a sample of 2 data (NOEC values) is considered to be sufficient (Aldenberg, personal communication), we feel that at least 3-4 NOEC values are needed, as the test organisms should represent various trophic levels, such as saprobes (decomposers), primary producers and consumers (Van Straalen and Denneman 1989).

For the aquatic environment, this could then consist of bacteria, algae, crustaceans, and fish. For our study no fish data were available, as they are not part of the routine effluent analysis carried out in our laboratory. The ETX extrapolation model has an advantage over other extrapolation models, such as those proposed by the USEPA (Stephan et al. 1985) and the OECD (1992), which require at least 8 test species.

Table 1. Some extrapolation constants for the calculation of one-sided left confidence limits for the logarithmic hazardous concentration for 5% of the species (HC5) on the basis of the logistic distribution; constants are tabulated for two levels of confidence: 95% and 50% or median (Aldenberg and Slob 1993).

m	95%	50%
2	27.70	2.49
3	8.14	2.05
4	5.49	1.92
5	4.47	1.85
10	3.06	1.73
20	2.49	1.68
50	2.10	1.65
100	1.95	1.64
500	1.76	1.63
∞	1.62	1.62

Application of the Model Using Effluent Data:

The current approach in the Netherlands in establishing safe levels for individual chemicals in the environment (water, sediments, soils) is using the ETX model. We have applied the ETX model, however, to effluent toxicity data. These effluent data were obtained as part of the evaluation program of 50 priority industries on the St. Lawrence (St. Lawrence Action Plan). A routine set of 4 bioassays were carried out within this program (Table 2). The SOS Chromotest results used in the model came from the biotest without S9 activation. It was felt that for the protection of the aquatic environment, mammalian liver microsome/cytosol activation of pre-genotoxic compounds was not relevant.

The ETX extrapolated values should be seen as the "overall" toxic potency of the effluent. NOEC values represent % vol/vol (effluent in the diluting medium), but the reciprocal (100/NOEC) is often used and expressed as Toxic Units (TU), which is dimensionless. The number of toxic units in an effluent sample for a particular biotest indicates the number of dilutions necessary to attain the NOEC level of that test. A type of "mass loading" (lets call it Toxicharge) of this overall/global toxicity (ETX-extrapolated TU's) in the effluent to the receiving waters can be calculated as follows:

$$\text{Toxicharge (TC)} = \text{TU} \times Q_E$$

where Q_E is the discharge or flowrate of the effluent (m^3/h). By calculating the TC values for each effluent, we can rank the industries, based not only on the toxic potency of their effluent, but also on the amount of "toxicity" being discharged to the receiving environment.

Table 2. Bioassay characteristics used for the toxicity evaluation of the industrial effluents.

Test/species	Organism	Trophic level	Toxic level	Toxic response
SOS Chromotest *Escherichia coli*	Bacterium	Decomposer	chronic sublethal	Genotoxicity
Microtox *Photobacterium phosphoreum*	Bacterium	Decomposer	acute sublethal	Light emission inhibition
Selenastrum capricornutum	Alga	Primary producer	chronic sublethal	Growth inhibition
Ceriodaphnia dubia	Crustacean (cladocera)	Primary consumer	chronic sublethal	Reproductive inhibition

An effluent discharged to a river is diluted, the extent and dynamics depending on the flowrate of the receiving waters. The dilution ratio (DR) can be stated as

$$DR = (Q_R + Q_E)/Q_E$$

where Q_R is the flowrate of the receiving waters. Note that it is not necessarily the flowrate of the whole river (we shall not consider a lake system), but rather that part with which the effluent is interacting. Thus, Q_R is a varying quantity, depending on the downstream distance from the effluent outfall.

Quebec provincial quidelines for effluent dilution and mixing zones to attain safe concentrations in the receiving waters are as follow (G. Duchesneau, personal communication): (1) maximum dilution allowed - 100x; and (2) maximum mixing zone - 300 m downstream or 1/2 width of river. The most limiting condition is taken as the allowable extent to which an effluent may be diluted to attain the no-effect concentration (which is in essence the NOEC level).

The concept of ETX extrapolated NOEC's can be combined with that of TU's, TC's, and DR's. The extrapolated NOEC is considered the level at which 95 % of the species are protected. Obviously, we cannot make a direct link between the laboratory surrogate species and those living in the receiving environment. Rather, the method is aimed at protecting most of the species in the community, but it is implicit that some species may be affected. This reflects somewhat the USEPA assumption that absolute protection of the most sensitive species is unnecessary or even impossible (Stephan et al. 1985).

The ETX-extrapolated NOEC is, therefore, used in a relative way. It will allow the ranking of the industries with respect to toxic potency of their effluents (TU) and their relative contribution to the receiving environment (TC), taking into consideration the rate of toxic emission. Furthermore, we can model the interaction of the the effluent with the receiving environment (DR) and extend this to establish the toxic mixing zone versus the "safe" no-effect zone, or at which distance downstream of the outfall we attain "no-effect concentrations".

With respect to the guidelines, we can now determine whether 100X dilution (or a 300m mixing zone) is sufficient to attain safe concentrations, and if not, what is the danger level (DL). The dimension of the latter can be determined as follows:

$$DL = TU/DR$$

where the TU's are based on the ETX NOEC values. Obviously, TU's of more than 100 are not acceptable according to the guidelines. However, depending on the river and the effluent flowrates, 100X dilution may not be achieved within the 300 m mixing zone (or 1/2 the riverwidth). Thus, DR will often be less than 100.

RESULTS AND DISCUSSION

For the purpose of illustrating the use of the ETX model, results for 3 industries are presented (Table 3). Note that the organic industry has been presented twice: first with the true effluent flowrate of 70 m^3/h, while second, with a hypothetical flowrate of 700 m^3/h. We hope to show in this way the influence of flowrate on the impact in the receiving waters. The same results are presented as TU in figure 3a ($\log_{10}$ values). ETX50 and ETX95 simply mean the median (50th percentile) and the 95th percentile estimates, respectively, as extrapolated by the ETX model.

Table 3. NOEC values (% vol/vol) of 4 bioassays for 3 industries, and ETX extrapolated NOEC values (median and 95th percentile).

Industry[1]	Q_{E}[2]	SOS	Microtox	S. Capr.	C. dubia	$NOEC_{ETX50}$	$NOEC_{ETX95}$
Organic-1	70	2	0.60	0.85	2.50	0.3428	0.0303
Inorganic	280	10	6.41	0.56	4.00	0.2990	0.0031
Paper	290	5	>100	40.00	0.18	0.0275	0.000001
Organic-2[3]	700	2	0.60	0.85	2.50	0.3428	0.0303

[1] Industries are: Organic - polymers, resins, plasticizers, herbicides; Inorganic - elementary phophorus; Paper - fine paper products.

[2] Effluent flowrate, $m^{3 \cdot 1}$

[3] Organic-2 is the same as Organic-1, only its flowrate has been hypothetically changed; see text.

The results for the organic industry shows that the individual NOEC values are fairly similar, resulting in ETX extrapolated values not too far removed. Obviously, the 95th percentile (ETX95) is more severe than the median estimate (ETX50). The values for the inorganic industry show more variability in the NOEC's, the algal test being more sensitive than the other tests. This results in a relatively low ETX95 NOEC (Table 3). Expressed as TU's (Fig. 3a), ETX95 indicates that this industry may discharge effluents with higher toxic potency than the organic industry, even though most of the bioassays indicated the contrary.

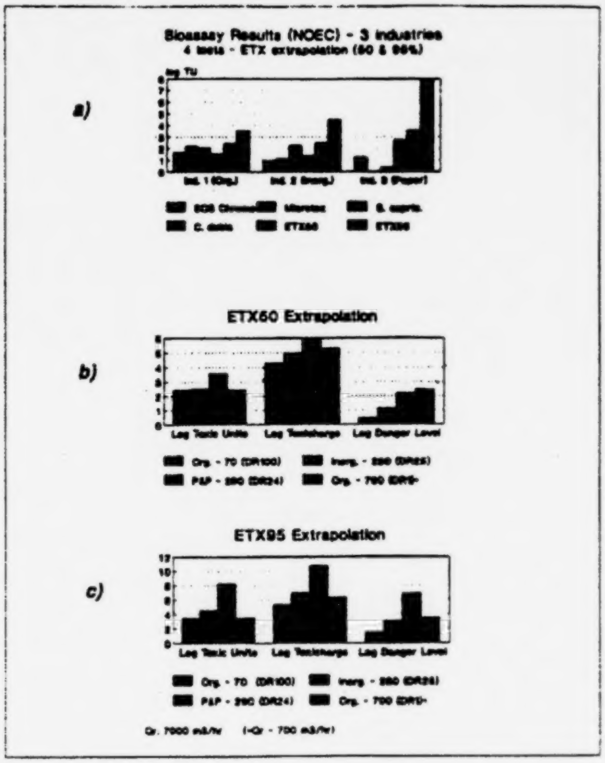


Fig. 3. Bioassay results, expressed as log Toxic Units (TU), for the organic, inorganic, and paper industry, including the ETX-extrapolated values, Toxicharge (TC), and Danger Level (DL).

The influence of having both very sensitive or very insensitive organisms to a particular effluent is quite evident for the paper industry. Microtox and the algal tests showed very low toxicity/sensitivity for its effluents (Table 3 and Fig. 3). The median ETX estimate is not that much affected, but the ETX95 estimate has become extremely high. The possibility that there might be highly sensitive organisms increases the uncertaintity about extrapolating "safe concentration" estimates. Thus, the ETX model provides an extra margin of safety for this high level of uncertaintity.

The extrapolated NOEC values can be regrouped to illustrate the relative toxic potency of the effluents (Fig. 3b and 3c). Log units are used to facilitate the presentation of widely different numbers. The organic industry with its hypothetical increased flowrate, but with the same toxic potency, as was explained before, has been included. Based solely on toxic potency, the organic and inorganic industries are similar, while the paper industry discharges a more toxic effluent. However, when we calculate the Toxicharge (TC), the inorganic industry contributes more toxicity to the receiving waters than the organic industry. This clearly shows that differences in flowrate can significantly influence the relative contribution of toxicity to the receiving environment. The Paper industry, however, still contributes the most toxicity.

The Danger Level (DL) was calculated using surrogate conditions with respect to the flowrate of the receiving waters. It was assumed that the effluent with the lowest flowrate (organic industry - 70 m^3/h) would attain a dilution ratio of 100 at the 300 m limit. From these conditions, the flowrate of the receiving waters at this downstream limit would then be 7000 m^3/h (the "true" mixing ratio would then actually be 101, but this slight difference is ignored). From this, the DR's of the other effluents can be easily calculated, by dividing their flowrates into the sum of effluent and receiving waters flowrates.

The relative DL's are presented in figure 3b and 3c, where the height of the bars indicates the "potential" danger impact or "risk" to the receiving environment for each industry. The organic industry represents the least danger to the environment. This is partly due to its low flowrate and "adequate" mixing within the 300 m zone. The situation for this industry is different when the effluent flowrate is much higher, hypothetically set at 700 m^3/h (not an unrealistic figure). Lets assume that the receiving river is much smaller, with an effective flowrate for mixing at the 300 m limit of only 700 m^3/h. In terms of Toxicharge (TC), the Paper industry still contributes the most toxicity to the receiving environment, but the organic industry is now second, at least for the median estimate or 50th percentile (Fig. 3b). However, the DL has now increased to the point that, based on the median estimate, this industry presents a greater risk to the receiving environment than the other two industries, even though the toxic potency of the latter two effluents are higher. Because of the high safety margin in the ETX95 estimate for the paper industry effluents, it remains the most important source of toxicity (Fig. 3c).

CONCLUSIONS

The ETX algorithm can be applied to effluent toxicity data to obtain an estimate of the global toxic potency. Two levels of confidence, the median and the 95 % estimate, are set for the extrapolation of NOEC values, below which 95% of the community, represented by the laboratory test species, are protected. Combining the global effluent toxicity and flowrate, industries can be ranked according to the Toxicharge (TC) value, which represents in essence a mass loading of Toxic Units (TU).

The physical interaction between the effluent and the receiving waters and the resulting lowering of toxicity through mixing and dilution can be simulated by calculating Dilution Ratios (DR) and Danger Levels (DL). Provincial (Quebec) guidelines specify a maximum allowable dilution of 100X to obtain no-effect levels. DL smaller than 1 indicates sufficient mixing, while values larger than 1 indicate insufficient dilution. The higher the DL value, the higher the risk the effluent presents to the receiving waters.

ACKNOWLEDGEMENT

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REFERENCES

Aldenberg, T. 1993. ETX 1.3a: A Program to Calculate Confidence Limits for Hazardous Concentrations Bases on Small Samples of Toxicity Data. National Institute of

Public Health and Environmental Protection (RIVM), Bilthoven, the Netherlands. 52p.

Aldenderg, T., and W. Slob. 1993. Confidence Limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicol. Environ. Saf. 25: 48-63.

Forbes, T.L., and V.E. Forbes. 1993. A critique of the use of distribution-based extrapolation models in ecotoxicology. Funct. Ecol. 7: 249-254.

Kooijman, S.A.L.M. 1987. A safety factor for LC50 values allowing for differences in sensitivity among species. Wat. Res. 21: 269-276.

OECD, 1992. Report of the OECD workshop on the extrapolation of laboratory data to the real environment. Organization for Economic Cooperation and Development (OECD) Environment Monographs No. 59. Paris, France. OCDE/GD(92)169.

Smith, E.P., and J. Cairns, Jr. 1993. Extrapolation methods for setting ecological standards for water quality: statistical and ecological concerns. Ecotoxicology 2: 203-219.

Sokal, R.R., and F.J. Rohlf. 1981. Biometry. The Principles and Practice of Statistics in Biological Research. 2nd edition. W.H. Freeman and Co., San Francisco.

Stephan, C.E., D.I. Mount, D.J. Hanson, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for deriving numeric National Water Quality Criteria for the protection of aquatic organisms and their uses. United States Environmental Protection Agency, Duluth, Minnesota. National Technical Information Service, PB85-227049.

Suter, G.W., L.W. Barnthouse, J.E. Breck, R.H. Gardner, and R.V. O'Neill. 1985. Extrapolation from the laboratory to the field: How uncertain are you?, p. 400-413. *In* R.D. Cardwell, R. Purdy, and R.C. Bahner [ed.] Aquatic Toxicology and Hazard Assessment. American Society for Testing and Materials, Philadelphia.

Van Straalen, N. M., and C.A.J. Denneman. 1989. Ecotoxicological evaluation of soil quality criteria. Ecotoxicol. Environ. Safe. 18: 241-251.

Wagner, C., and H. Lokke. 1991. Estimation of ecotoxicological protection levels from NOEC toxicity data. Wat. Res. 25: 1237-1242.

ECOLOGICAL VERSUS STATISTICAL SIGNIFICANCE. B. Kilgour.

No abstract available.

SURVIVAL ANALYSES OF RAINBOW TROUT (*Oncorynchus mykiss*) EGG HATCHABILITY DATA AND ENVIRONMENTAL DECISION MAKING.

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ABSTRACT

To determine the environmental impact of point and non-point discharges to the St. Clair River an in-situ biomonitoring program, using rainbow trout (*Oncorhynchus mykiss)* eggs was conducted annually between 1989 to 1992. The per cent egg survival, and time to death were analyzed using logistic regression and survival analysis respectively, with both techniques indicating a significant discharge effect. The inclusion of the variable, daily rainfall in the survival analysis, negated the station effect in half of the years studied, implying that non-point sources such as urban, industrial and agricultural run-off may play an important role in the response observed. The use of statistical models capable of including time-dependent variables, may provide a better understanding of in situ bioassays than conventional methods.

INTRODUCTION

Survival analysis refers to a broad class of statistical models, which model the time to mortality or failure, as a function of explanatory variable(s). Since these models are inherently temporal in nature, variables which affect the overall mortality by acting on the daily-specific mortality can be included. Statistical techniques such as Analysis of Variance (ANOVA) or logistic regression do not usually include variables indexed by time.

The Lambton Industrial Society (LIS) commissioned an *in situ* bioassay investigating the collective effects of effluent discharges on the aquatic biota of the St. Clair River using an early life stage with Rainbow Trout eggs. Experimental stations are shown in Fig. 1. The data was originally analyzed using ANOVA and survival analysis to determine the time to 25\% mortality. The data was reanalysed using survival analysis and logistic regression, both with and without the variable rainfall.

MATERIALS AND METHODS

The experimental design was a 2x2 factorial with 12 replicates at each factor combination. The factors were station, (upstream and downstream) (Refer to Fig. 1) and water filtration (either filtered (down to 5 µm), or unfiltered). Approximately 200 eggs were placed in each incubation chamber resulting in an exposure of 2 stations X 2 filtration regimes X 12 replicates X approximately 200 eggs/tank = approximately 9600 eggs. This design was repeated over 4 years, beginning in 1989 and ending in 1992. The experiment was begun at different times in the spring of each year in an attempt to expose the eggs to the same temperature regime. Initial dates for the years 1989 to 1992 were May 16, May 29, May 22 and May 20, respectively with initial upstream temperatures of 10.0, 13.5, 14.0, and 9.4°C, respectively. For a more detailed discussion of materials and methods refer to Moran et al. (1993).

The data was analyzed using SAS (SAS Institute Inc., Cary, N.C. 27512-8000). The first model analyzed was % mortality = station + year + filtration, using logistic regression. Then the model time to mortality = station + year + filtration, was fit using an exponential survival model. This same model was fit again with the addition of the variable, daily rainfall.

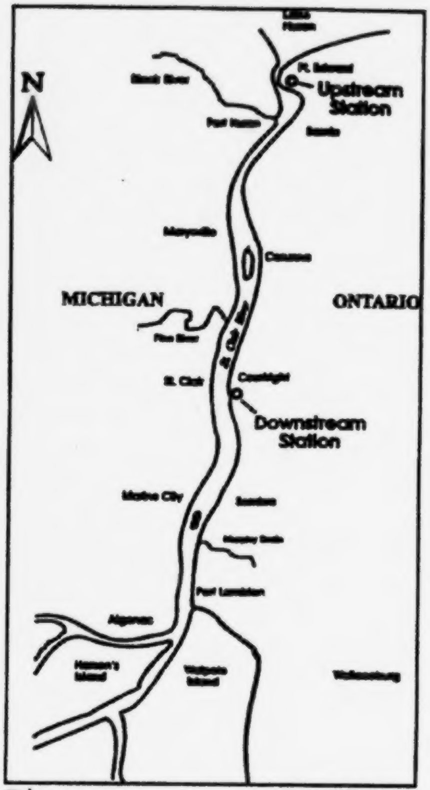

Figure 1: Sampling locations on the St. Clair River, Ontario, Canada.

RESULTS

The logistic regression and the survival analysis (without rainfall) showed that the effect of station was significant in all years. However when the variable rainfall was added to the survival model, the effect of station was negated in two of the years (1989 and 1992). The significance of the variable filtration remained consistent throughout all analyses.

DISCUSSION

In this example, both survival analysis and logistic regression showed that there was a significant difference in survivorship between stations. However the addition of the variable rainfall, negated the effects of station. The change in significance of location in half the years studied due to the inclusion of the variable rainfall may lead regulators to question whether the effluent discharges are impacting aquatic biota.
The inclusion of the lurking variable rainfall tends to indicate that non-point sources such as urban and agricultural run-off may play an important role in down-stream toxicity. Atrazine levels increased after a rainfall event in 1992, lending credence to this alternative hypothesis.

REFERENCE

Moran, T., M. Lines, K. Getty and T. Kierstead. 1993. *In situ* multi-species approach to environmental effects monitoring of a major river ecosystem. Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop. October 4-7, 1992, Edmonton, Alberta. Can. Tech. Rep. Fish. Aquat. Sci. 1942: 489 p.

SOIL TOXICOLOGY
SESSION CHAIR: C.D. WREN

WORM BIOASSAYS: WHAT KNOWLEDGE CAN BE APPLIED FROM AQUATIC TOXICITY TESTING?

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ABSTRACT

Current dogma suggests that the effective concentration of soil contaminants is that fraction that resides in the hydrosphere of soil particles. Since much of the toxicity is due to the waterborne fraction in the soil, parallels may be drawn between the toxicity of soil, sediment, and waterborne contaminants. One distinct difference is that the bioavailable fraction of contaminants in soil and sediment is extremely difficult to determine by analytical chemistry, whereas in a water matrix, chemical analysis and prediction of speciation, and hence bioavailability, is often simpler. Basic concepts of aquatic toxicity testing applied to the soil medium should increase the general understanding of the toxicity of soil contaminants. The use of lethality thresholds or incipient lethal levels (ILLs) would increase the accuracy of the estimation of toxicity and kinetics in varying soil types. The application of toxicant kinetics and critical body residues (CBR) in relation to toxicity endpoints may provide a more suitable estimate of dose, within the dose-response framework, than the soil levels of contaminants, especially for hydrophobic organic chemicals. Body residues in test organisms also provide a useful tool for examining the bioavailability of soil contaminants and enhancing our understanding of the toxicity of chemical mixtures in soils. These concepts can be readily incorporated into the general framework of existing earthworm bioassay protocols and their application is fundamental to toxicity testing in soil systems.

INTRODUCTION

While research has been conducted with various species of worms in both natural and artificial soils (Goats and Edwards 1988, Edwards and Bohlen 1992) to determine the biological effects of agricultural chemicals on non-target species, it has only been in the latter part of the last decade that the demand for such information has increased dramatically. Much of this attention is a direct result of the increased interest in the identification and remediation of contaminated sites and the need for data linking invertebrate food sources with higher trophic levels for purposes of environmental risk assessment.

Soil toxicity testing is similar in nature to the testing of contaminated marine and freshwater sediments, thus it is also subject to many of the same problems. As a result of the regulatory and/or legal basis for the increase in soil toxicity testing, much of the work is focused on very specific objectives such as "Are contaminant concentrations in the soil at a site below the concentration specified for each contaminant in the regulation?" or "Is this soil toxic?" Toxicity testing for such regulatory purposes is usually directed towards site-specific objectives, such as determining if any toxic effects occur, and not understanding the underlying causes and mechanisms of toxicity. For example, contaminated site cleanup is often an exercise in meeting soil quality objectives or guidelines, with no site-specific toxicity testing and/or toxicological interpretation. Valuable information is often lost in the rush to answer a few very specific questions of limited scope and value. More importantly, the testing procedures may not be sufficiently rigorous to confirm fulfilment of fundamental assumptions involved in test design. Such approaches may initially save a relatively small amount of time and money, but provide less general scientific information to advance toxicological understanding, and often leave the interpretation of test results, and any conclusions based on them, open to scientific and legal challenges.

Soil toxicity testing is in the process of being rigorously investigated, with protocols and procedures being codified. These tests may benefit substantially from the knowledge obtained during the development of aquatic toxicity tests. Although neither of these areas could be considered to be scientifically mature, aquatic toxicity testing has been more intensively studied for a longer period of time. This paper will examine a number of areas where aquatic toxicologists have studied problems germane to soil toxicity testing and recommendations for soil testing will be made using earthworm toxicity tests as an example.

Although the terms toxicity test and bioassay are often used interchangeably, bioassay is the more general of the two, and toxicity tests should be considered a more specific case of the general term. According to Finney (1978) "A biological assay (or bioassay) is an experiment for estimating the nature, constitution, or potency of a material (or of a process), by means of the reaction that follows its application to living matter." A bioassay involves a stimulus of a measured external exposure or absorbed dose which is applied to a subject whose response to the stimulus is estimated in the change of some biological characteristic or state of the subject. In soil toxicity tests, only the bioavailable fraction of a chemical present in the soil which comes in contact with and enters the body of the exposed organism, and ultimately the fraction of the absorbed dose which reaches the site(s) of toxic action, is associated with adverse effects. Hence, toxicity is not a fixed physical/chemical property of a soil, such as temperature or pH, rather it is an adverse biological response which results from the interaction between the soil and the organism. Adverse or toxic responses occur when soil-based exposure, and the resultant soil/organism interaction result in a sufficient dose of chemical at the site of toxic action within the organism. Organisms, and only organisms, can determine bioavailability and toxicity; chemical analysis of soils measures only chemical concentrations in soil. The chemical concentration in the body of an exposed organism is a far better surrogate for the dose at the site(s) of toxic action in the organism than an external estimate such as the concentration of chemical in soil.

For these reasons, soil quality guidelines can be under- or overprotective for the toxicity of chemicals in soils, depending on the quality of the bioassays that are employed in guideline development, and on the quality of toxicological interpretation of bioassay results. As soil-based bioassays have much in common with aquatic bioassays, soil quality guidelines are closely related and are subject to the same criticisms as water quality guidelines. As is the case for many water quality guidelines, established guideline or objective levels for soil contaminants often do not take into account naturally occurring geographic variation in contaminant levels, synergistic effects of chemical mixtures, or sublethal effects such as growth and reproduction. Current soil quality guidelines are usually based upon lethality data derived from tests with soil organisms using soil chemical concentrations with little or no knowledge of corresponding chemical concentrations in the organism. Given the parallels between soil and aquatic systems, and the much larger information resource of aquatic bioassay results, it should be instructive to compare and contrast the two with the objective of saving soil toxicity testing some of the problems and errors already experienced by aquatic bioassay practitioners.

Bioassay Design

Exposure Systems: Typical worm bioassays are the equivalent of static aquatic bioassays, the least desirable method of exposure available, and subject to many difficulties such as depletion of chemical (evaporation, biotransformation, photolysis etc.), alteration of soil characteristics with time, depletion of food available to the test organisms, and contamination of the test medium with metabolic by-products. The exposure regime selected will affect not only the analysis and interpretation of the results, but the underlying assumptions for the tests. Many contamination events are more continuous in nature, with a constant or repeated addition of a contaminant over time. Currently, there are no standard methods for conducting a continuous exposure in soil toxicity tests, and standard protocols represent a single or pulse contamination of soil. Even partial solutions such as media replacement methods have the problem of frequent disturbance of the test organisms with a resultant increase in stress. One improvement in the current static methodology would be to increase the number of replicates which can be sampled at discrete times in a logarithmic time series. Furthermore, to overcome the declining exposure concentration scenario, a shift from soil concentration-based assessment to organism residue-based assessment could be readily developed from existing knowledge of soil chemistry, earthworm physiology, physical and chemical properties of contaminants, and toxicokinetic modelling.

Choice of Testing Medium: The choice of test medium for soil bioassays depends on the purpose or objectives of the test. You may be trying to either replicate site-specific conditions or maximize toxicological interpretation and understanding. Most often the former objective is desired when the latter is more appropriate. Toxicity tests are usually conducted in the laboratory using a standardized artificial soil to reduce variability in estimates of the toxicity of soil contaminants to worms. Artificial soils and filter-paper tests are used because bioavailability is highly variable in natural soils (Lanno et al. 1994, van Gestel and Ma 1988, 1990). Although the use of artificial media allows for a standardized comparison of the toxicity of various chemicals to a test organism, accurately extrapolating to real world situations with different species and chemical mixtures is difficult. Another option that has been explored recently is the exposure of terrestrial oligochaetes in aqueous systems (Belfroid et al. 1993a,b). These researchers examined the bioconcentration kinetics and lethality of chlorobenzenes to *E. fetida* in an aqueous environment. Worms survived 29 days of aqueous exposure without feeding. This exposure regime offers an alternative or a prelude to soil exposures, when certain assumptions are to be met for modelling purposes. A third method of exposing worms to contaminants is via the diet. Many different methods have been tried, such as forced-feeding using a syringe (Edwards and Bohlen, 1992), or feeding chemically-treated food to the worms. The latter method shows some promise and appears to be have used successfully by Belfroid et al. (1993a). It may allow the incorporation of the feeding biology of the test species into the exposure regime by offering the food on the surface of the soil, as a central plug, or homogeneously mixed into the soil. Many of the problems encountered in feeding and quantifying feed intake during aquatic toxicity tests (Lanno et al. 1989), such as differentiating between toxicant effects and the effects of reduced food consumption, are also common to earthworm toxicity tests.

Exposure Conditions: The testing of contaminants in a soil matrix offers a unique advantage over testing in an aqueous medium: chemicals which do not readily dissolve in water can be applied directly to the soil in liquid or powder form. Solvent carriers, such as acetone, are common to both aqueous and soil toxicity tests, but they have the disadvantage of altering the bioavailability. This may be counterproductive to understanding the bioassay unless specific information on accumulation (i.e., such as body residues) is also collected.

E. fetida has been adopted as the standard organism for earthworm toxicity tests (ISO 1991, Greene et al. 1989). However, regulatory agencies may recommend the use of an indigenous species for toxicity tests. For example, Environment Canada has shown some interest in the use of *Lumbricus terrestris* in toxicity tests, as it may be more representative of environmental conditions in Canada than *E. fetida*. Adapting the standard protocol for use with *L. terrestris* results in a number of problems associated with body size scaling and species effects which would need to be addressed.

The choice of endpoints for a toxicity test may affect its relevance for application in the assessment of environmental health. All laboratory end-points have a limited capacity for extrapolation to higher levels of biological organization because they reflect only the average response of a selected group of organisms within a given species, and they are not designed to include the multiple interactions necessary for integration with higher levels of biological organization. Mortality, growth, and reproduction are endpoints that are commonly included in population models. Biomarkers of chemical exposure, such as neurophysiological responses or the induction of mixed-function oxidase (MFO) enzymes, are of little use in terms of ecological relevance unless they can be linked to mortality, growth, or reproduction.

Chemical mixtures: Very few situations occur in the real world where worms would be exposed to a soil contaminated with a single chemical. As an example, soils contaminated with wood preservatives may contain mixtures of compounds such as pentachlorophenol (PCP), polycyclic aromatic hydrocarbons (PAHs), and their metabolites. As in aquatic toxicity testing, one of the most difficult challenges is to assess the toxicity of chemical mixtures. Many methods for the assessment of mixture toxicity have been attempted in aquatic toxicity testing, such as the Toxic Unit Concept (Sprague 1970), but recent advances in theory suggest the various body residues associated with chemical of different modes of toxic action may offer an improved method for measuring and interpreting the toxicity of many chemical mixtures, especially for organic chemicals (McCarty and Mackay 1993).

Modifying Factors

Abiotic and biotic factors that influence toxicity are generally described as *modifying factors* and may be factors related to exposure (time, pH, temperature), the organism (species, behaviour, size), or the chemical itself (solubility, lipophilicity, impurities)

(Sprague 1985). In sediment toxicity testing, the debate rages on as to whether the bulk-phase concentration or the porewater concentration of a contaminant is the best surrogate measure for the toxicity of a chemical. A similar argument could be developed for soils, although it would be extremely difficult to extract sufficient pore water from many soils to conduct tests and the interpretation of the results would be difficult. Most of these modifying factors influence bioavailability and partitioning and their influence could be accounted for and, in fact, quantified using body residues.

There is a substantial database on the feeding and growth of earthworms, particularly *E. fetida* (Lee 1985). One of the major questions once associated with acute toxicity tests in aquatic systems was whether or not to feed the test organisms during the tests, and if so, how to feed them (Lanno et al. 1989). The standard recommendation for an acute toxicity test with fingerling rainbow trout is to not feed them unless the test duration is extended beyond 96 h (Sprague 1973). This recommendation is species-specific and, in addition to test duration, will vary with the body size and life history stage (e.g. age) of the test organism. Fish can be separated easily from their food in test systems simply by withholding feed. Soil toxicity tests with worms do not always allow the same luxury, since the test medium is often ingested by the test organism and it will derive some nutritive value from it. Sphagnum moss appears to have low nutritive value, and growth during a 14-d test with *E. fetida* will be limited. Beyond the 14-d test, growth may be reduced, so in order to maintain a positive energy balance in the test organism, it may be necessary to provide some source of food.

The addition of food has its own inherent problems such as the binding of the test compound by food and the presence of chemicals in the food itself that may alter the toxicological response of the test organism. An improvement in the artificial soil recommended for toxicity tests is the addition of some form of digestible organic matter for conducting toxicity tests that extend beyond 14 d. This should be standardized by monitoring the growth rates of worms over a 28-d period with various levels of different organic matter to ensure that no weight loss occurs. An additional problem encountered with soil toxicity tests is the inability to differentiate between dietary and cuticular routes of contaminant uptake; toxicity estimates obtained via exposure-based testing may vary greatly with different routes of exposure. This has been noted in fish, but Belfroid et al. (1993a) suggest based upon lethal body burdens that the toxicity of chlorobenzenes to *E. fetida* is approximately the same by dietary and cuticular routes of uptake.

The fate of organic test chemicals in soil would appear to be much more complex than in water. Microbes that are inherent in soil environments could metabolize parent compounds to innocuous byproducts or transform them to more potent intermediary metabolites. Additionally, the absorption, metabolic degradation, and sequestration of many organic chemicals by worms is poorly understood. Much more information is available on the absorption, compartmentalization, and metabolism of metals in earthworms (Morgan et al. 1993).

The lipid content of a test organism is an important modifying factor in toxicity testing and should always be reported. Common practice in some aquatic bioassays, especially bioconcentration/bioaccumulation studies, is to normalize data from tests with organic chemicals to organism lipid content. Although useful for these purposes, it may be problematic for toxicological purposes as the ratio of total body lipid to target site lipid likely varies widely between species and within species with differences in sex, age, and nutritional state. An excellent example of the correct toxicological application of lipid content is presented by Geyer et al. (1990). Differences between LD50 values for tetrachlorodibenzodioxin (TCDD) in mammals ranging over 4 orders of magnitude were explained by differences in body fat ranging from about 5-20% (log LD50 = 5.30 * log total body fat - 3.22; r^2=0.834, n=20) (Geyer et al. 1990); the more fat the less toxic TCDD appeared to be. The lipid content of worms appears to be quite low relative to fish. The total lipid of many species of worms (*Eisenia andrei, Lumbricus spp., Aporrectodea caliginsoa tuberculata*) has been found to range from 1 to 3% (Belfroid et al. 1993b, 1994, Knuutinen et al. 1990, Albro et al. 1992, Haimi et al. 1992), while the total lipid content of rainbow trout used in toxicity tests is usually in the range of 5-8% (van den Heuvel et al. 1991). Albro et al. (1992) reported the lipid class composition of *L. terrestris* to be 29% simple lipids (including glycerides), 59% phospholipids, with glycolipids comprising the remainder. No information was available on the relationship between lipid classes and bioaccumulation or toxicity in earthworms.

Analysis and Interpretation of Results

Toxicity Curves: Lethality tests with earthworms typically involve measuring worm mortalities at 7 and/or 14 days, which is substantially longer than the 4-d (96-h) duration typical of aquatic toxicity tests with fish. This temporal regime may often be sufficient to reach a time-independent threshold or incipient lethal level (ILL) (Lanno et al. 1994; Fitzgerald et al. 1994). This depends upon the test organism, the test medium, and the kinetics of the contaminant being examined. A comparison of toxicity curves for diazinon in aquatic toxicity tests conducted with fathead minnows and toxicity curves for *L. terrestris* exposed to diazinon in three natural soils is presented in Fig. 1.

The 14-d exposure regime suggested for earthworm lethality tests appears to be of sufficient duration to achieve a lethality threshold with many of the chemicals of concern that have been tested, and in fact, 7 days is adequate. However, caution should be taken when tests are conducted with extremely hydrophobic chemicals (PCBs, dioxins, some pesticides), as a 14-d exposure may not be sufficient to achieve a lethality threshold for either fish (Niimi 1987) or earthworms. In Fig. 1, two of the worm bioassays reached the ILL during the 14 day exposure but the third (Fox sand) did not as steady-state is usually considered to be reached after 4-5 half-lives (corresponding to 94-97% of the ultimate goal). Karnak and Hamelink (1982) exposed *L. terrestris* to benomyl and estimated 7- and 14-d LC50s of 1.70 to 0.42 mg/kg, respectively. The significant decrease in LC50 values between 7 and 14 days suggests that a lethality threshold had not been established by 14 days and the test duration should have been extended.

The ILL is a fundamental concept in toxicity testing (Sprague 1969) that is often ignored, even in aquatic toxicity testing. It is defined as that concentration of a contaminant at which half of the exposed population will survive indefinitely and is estimated from a toxicity curve. The ILL acts to normalize the toxic response so that all comparisons of toxicity within and between chemicals and species of organisms are made at a steady-state with respect to the dose-response curve. In order to estimate the ILL by applying various models, a minimum number of observations are necessary, with two not being sufficient to define the curve. The ideal solution is to follow Sprague's advice by not using a fixed exposure duration and continuing observations until the ILL is established. Barring

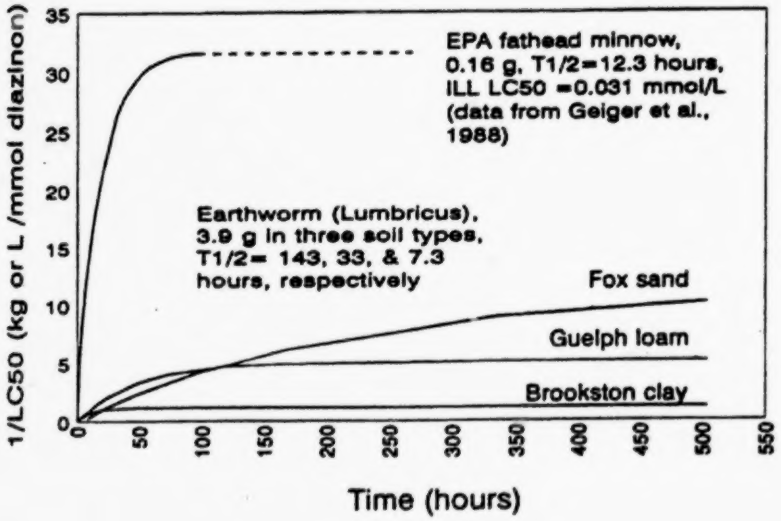
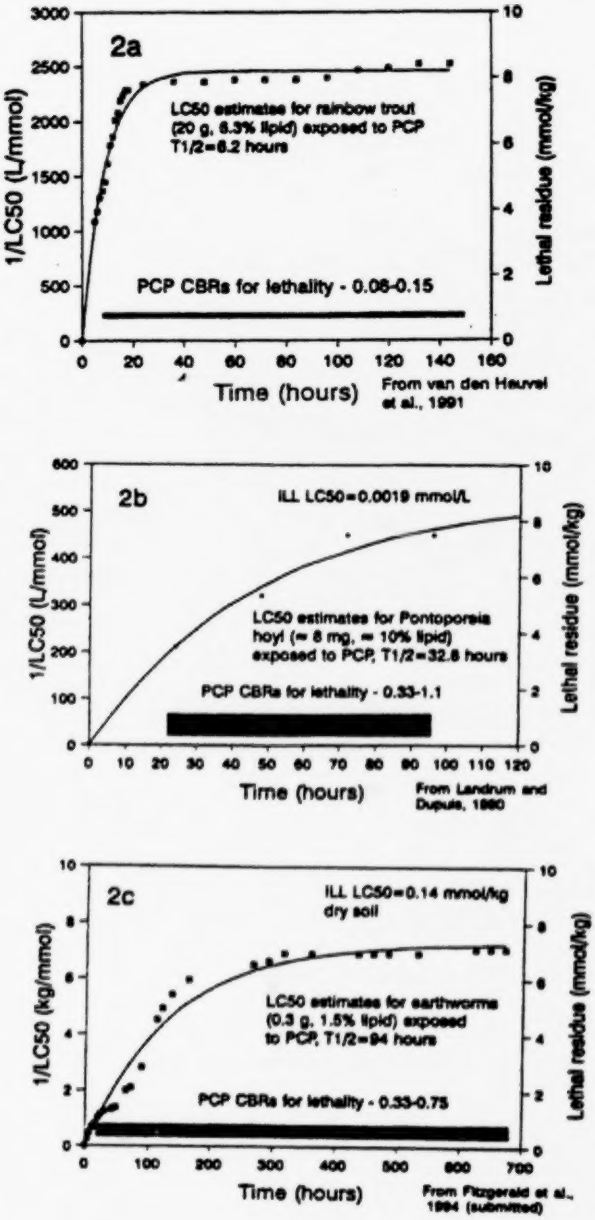


Fig. 1. A comparison of toxicity curves for the pesticide diazinon in toxicity tests conducted with fathead minnows in water and *L. terrestris* exposed to diazinon in three natural soils.

this, the accuracy and utility of soil toxicity estimates could be improved by increasing the number of observations and redistributing observation times with more frequent observations being made early in the test, as typified by the logarithmic sequences suggested by Sprague (1973). For example, with observations taken at 0.5, 1, 2, 4, 8, and 14 days, simple modelling techniques similar to those used for fish and aquatic invertebrate bioassays could be used to estimate the timing and value of the ILL (Landrum and Dupuis 1990, van den Heuvel et al. 1991, McCarty et al. 1992). Although more frequent observations may present technical difficulties (ie. disturbance of worms and test medium), replication at each observation time may circumvent logistical and statistical problems.

Body Residues and Effects: The bioavailability of a chemical can be dramatically altered by characteristics of the test soil (Fig. 1) such as particle size, pH, and organic matter content. Body residues should assist in both assessing bioavailability and improving the understanding of the dose associated with the response pattern observed during a test. Belfroid et al. (1993b) observed that lethal body burdens in *E. fetida* were similar to those of small fish for 1,2,3-trichloro- and pentachlorobenzene (0.5-2 mmol/g wet weight). As illustrated in Fig. 2, similar CBRs were also found to exist for fish, earthworm, and aquatic crustacean toxicity (0.082-0.165, 0.33-0.79, and 0.3-1.1 mmol/kg wet weight) resulting from exposure to pentachlorophenol (van den Heuvel et al. 1991, Fitzgerald et al. 1994, Landrum and Dupuis 1990). A discussion of the theory and practice of residue-effect relationships for aquatic organisms is presented in McCarty et al. (1992) and McCarty and Mackay (1993).



From van den Heuvel et al., 1991

From Landrum and Dupuis, 1990

From Fitzgerald et al., 1994 (submitted)

Fig. 2. LC50 estimates for pentachlorophenol toxicity versus exposure time for rainbow trout (2a), the aquatic amphipod *Pontoporeia hoyi* (now *Diporeia spp.*)(2b), and the earthworm *E. fetida* (2c). Bars represent the range of critical body residues (CBRs) at mortality.

SUMMARY AND RECOMMENDATIONS

The obvious parallels between toxicity tests conducted in water, sediments and soil allows us to combine the expertise developed in these discrete disciplines. Fundamental concepts that have been applied in aquatic toxicity testing, and often forgotten in the regulatory rush, can be applied easily to toxicity tests conducted in soils. The 14-d exposure regime often used in earthworm tests appears to be of sufficient duration to achieve a lethality threshold with many chemicals of concern. However, for some very hydrophobic chemicals (PCBs, dioxins, some pesticides) and for various chemicals in certain types of soil, a 14-d exposure may be insufficient for a lethality threshold to be reached. Furthermore, more frequent observations are needed as an experimental duration of 14 days or longer with observations about every 7 days is insufficient for the estimation of the kinetics or the determination of a lethality threshold or incipient lethal level (ILL).

The use of body residues in the assessment of environmental contaminant exposure and toxicity is receiving increased attention in aquatic toxicity testing and ecotoxicology (McCarty and Mackay 1993). Since the relationship between bioavailability and measured levels of chemicals in soils differing in physical and chemical properties is often obscure, advances in analytical capabilities may be used to determine chemical levels in soil test organisms. Body residues may provide a more direct and toxicologically relevant estimate of the actual internal dose of the exposed organism and also provides a better basis for comparison of test data from different test media, such as artificial soils, natural soils, diet, and water. By estimating both soil and organism chemical levels, differences in bioavailability can be readily identified and investigated. Although residues have been used for some time in assessing the of exposure of worms to various pesticides in the field (Edwards and Bohlen 1992), tremendous benefit may be derived by relating residues in both field and laboratory studies to toxicity endpoints such as growth, reproduction, or population structure.

Clearly, the adoption of two general recommendations would substantially increase the scientific validity and utility of current soil toxicity test protocols: (1) Regardless of the exposure duration, frequent observations (≥ 5), preferably in a logarithmic sequence, should be made, and these data should be used to estimate and report both the kinetics (i.e., half-life) and the time-independent, steady-state, exposure concentration or incipient lethal level (ILL); and (2) whole-body concentrations of the chemical being tested should be measured in the control and exposed organisms, especially those that have died. This data can be used to estimate toxicant kinetics and critical body residues (CBR) for the chemical and organism being studied.

REFERENCES

Albro, P.W., J.L. Schroeder and J.T. Corbett. 1992. Lipids of the earthworm *terrestris*. Lipids 27: 136-143.

Belfroid, A., M. Sikkenk, W. Seinen, K. van Gestel and J. Hermens. 1994. The toxicokinetic behaviour of chlorobenzenes in earthworm (*Eisenia andrei*) experiments in soil. Environ. Toxicol. Chem. 13: 93-99.

Belfroid, A., Seinen W., van Gestel K. and J. Hermens. 1993a The acute toxicity of chlorobenzenes for earthworms (*Eisenia andrei*) in different exposure systems.

Chemosphere 26: 2265-2277.

Belfroid, A., A. van Wezel, M. Sikkenk, K. van Gestel, W. Seinen and J. Hermens. 1993b. The toxicokinetic behaviour of chlorobenzenes in earthworms (*Eisenia andrei*): experiments in water. Ecotox. Environ. Safety 25: 154-165.

Edwards, C.A., and P.J. Bohlen. 1992. The effects of toxic chemicals on earthworms. Rev. Environ. Contam. Toxicol. 125: 23-99.

Finney, D.J. 1978. Statistical Method in Biological Assay. 3rd edition. C. Griffin and Co. Ltd., London.

Fitzgerald, D.G., R.P. Lanno, U. Klee, A. Farwell and D.G. Dixon. 1994. Critical body residues (CBRs) in assessing the toxicity of pentachlorophenol to *Eisenia fetida*. Submitted Soil Biol. Biochem.

Geiger, D.L., D.J. Call and L.T. Brooke L.T. (Ed.). 1988. Acute Toxicities of Organic Chemicals to Fathead Minnows *Pimephales promelas*. Vol. 4. University of Wisconsin-Superior, Superior, Wisconsin. 355 p.

Geyer, H.J., I. Scheunert, K. Rapp, A. Kettrup, G. Korte, H. Greim and K. Rozman. 1990. Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and total body fat content in mammals. Toxicology 65: 97-107.

Goats, G.C., and Edwards C.A. 1988. The prediction of field toxicity of chemicals to earthworms by laboratory methods, 283-294. *In* Earthworms in Waste and Environmental Management, C.A. Edwards and E.F. Neuhauser (Ed.), Academic Publishing, Hague, Netherlands.

Greene, J.C., C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, S.A. Peterson and W.E. Miller. 1989. Protocols for Short Term Toxicity Screening of Hazardous Waste Sites. US EPA, EPA 600/3-88-029.

Haimi, J., J. Salminen, V. Huhta, J. Knuutinen and H. Palm. 1992. Bioaccumulation of organochlorine compounds in earthworms. Soil Biol. Biochem. 24: 1699-1703.

ISO. 1991. Soil quality - Effects of pollutants on earthworms (*Eisenia fetida*). Part 1: Method for the determination of acute toxicity using artificial soil substrate. International Standards Organization.

Karnak, R.E., and J.L. Hamelink. 1982. A standardized method for determining the acute toxicity of chemicals to earthworms. Ecotox. Environ. Safety 6: 216-222.

Knuutinen, J., M. Palm, H. Hakala, J. Haimi, V. Huhta and J. Salminen. 1990. Polychlorinated phenols and their metabolites in soil and earthworms of sawmill environment. Chemosphere 20: 609-623.

Landrum, P.F., and W.S. Dupuis. 1990. Toxicity and toxicokinetics of pentachlorophenol and carbaryl to *Pontoporeia hoyi* and *Mysis relicta*. *In* Aquatic Toxicology and Risk Assessment, Thirteenth Volume, W.G. Landis and W.H. van der Schalie, (Ed.), American Society for Testing and Materials, Philadelphia PA.

Lanno, R.P., C.D. Wren and G.L. Stephenson. 1994. The use of toxicity curves in assessing the toxicity of soil contaminants to *Lumbricus terrestris*. Submitted Soil Biol. Biochem.

Lanno, R.P., B.E. Hickie and D.G. Dixon. 1989. Feeding and nutritional considerations in aquatic toxicology. Hydrobiologia 188/189: 525-531.

Lee, K.E. 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use. Academic Press, New York.

McCarty, L.S., D. Mackay, A.D. Smith, G.W. Ozburn, and D.G. Dixon. 1992. Residue-

McCarty, L.S., and D. Mackay. 1993. Enhancing ecotoxicological modelling and assessment: body residues and modes of toxic action. Environ. Sci. Technol. 27: 1718-1728.

Morgan, A.J., J.E. Morgan, M. Turner, C. Winters and A. Yarwood. 1993. Metal relationships of earthworms, p. 333-358. *In* Ecotoxicology of Metals in Invertebrates, R. Dallinger and P.S. Rainbow (ed.), Lewis Publishers, Boca Raton, FL.

Niimi, A.J. 1987. Biological half-lives of chemicals in fishes. Rev. Environ. Contam. Toxicol. 99: 1-46.

Gobas, F.A.P.C., K.E. Clark, W.Y. Shiu, and D. Mackay. 1989. Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: role of bioavailability and elimination into the feces. Environ. Toxicol. Chem. 8: 231-245.

Sprague, J.B. 1985. Factors that modify toxicity, p. 124-163. *In* Fundamentals of Aquatic Toxicology, G.M. Rand and S.R. Petrocelli (ed.), Hemisphere Publishing Corporation, Washington, DC.

Sprague, J.B. 1973. The ABCs of pollutant bioassay with fish, p.6-30. *In* Biological Methods for the Assessment of Water Quality, STP 528, J. Cairns Jr. and K.L. Dickson (ed.), American Society for Testing and Materials, Philadelphia, PA.
Sprague, J.B. .1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4: 3-32.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

van den Heuvel, M.R., L.S. McCarty, R.P. Lanno, B.F. Hickie, D.G. and Dixon. 1991. Effect of total body lipid on the toxicity and toxicokinetics of pentachlorophenol in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 20: 235-252.

van Gestel, C.A.M., and W.C. Ma. 1990. An approach to quantitative structure-activity relationships (QSARs) in earthworm toxicity studies. Chemosphere 21: 1023-1033.

van Gestel, C.A.M., and W.C. Ma. 1988. Toxicity and bioaccumulation of chlorophenols in earthworms in relation to bioavailability in soil. Ecotox. Environ. Safety 15: 289-297.

COMPARISON OF SELECTED TEST METHODS FOR SCREENING SOIL CONTAMINANTS. D.R. Lintott[1], R. Lifshitz[2], K. Poirier[1] and S. Goudey[1]. [1]HydroQual Laboratories Ltd., #3-6125 12th St. S.E., Calgary, AB, T2H 2K1; [2]Environmental Bio-detection Products Inc., 14 Abacus Rd., Brampton, ON, L6T 5B7.

Soil toxicity screening tests typically involve extraction with various solvents and testing the extract with aqueous toxicity tests. The efficiency of extraction procedures and liquid-phase testing to identify a broad range of toxins is questionable. Bound residues, which may not be removed by extraction, are of particular concern. The ability of direct solid-phase screening tests to identify toxicity in contaminated soils was examined and compared to routine extraction and testing procedures. Toxicity tests included solid- and liquid-phase bacterial luminescence (Microtox) and Toxi-chromotest. The solid-phase tests allow the test organisms to come in direct contact with toxicants bound to particulate matter in an aqueous suspension. The test samples included artificial soils spiked with mercury, chromium (VI), arsenic, pentachlorophenol, 2-chloroacetamide and ethylene glycol, along with three contaminated soils from a gas plant site. Tests were done on the solid matrix, methanol and aqueous extracts, and on the pure compounds in water. The solid-phase methods measured the total effective toxicity of the solid material, detecting insoluble as well as soluble organic and inorganic toxicants. In contrast, the toxicity of extracts of contaminated soils were not as reliable an indicator of toxicity, due to incomplete extraction.

A BIOLOGICAL CLEANUP STRATEGY FOR HAMILTON HARBOUR SEDIMENTS. T.P. Murphy and A. Moller. National Water Research Institute, Environment Canada, Burlington, ON, L7R 4A6.

To support the remedial action plan of Hamilton Harbour and to determine the extent of coal tar contamination in a toxic area of the harbour, >100 sediment cores and >100 sediment Ekman grab samples were collected for biological and chemical study. The concentration of polynuclear aromatic hydrocarbons (PAHs) that results in the death of 50% of the *Daphnia magna* and *Hexagenia* is less than 24 µg/g and 329 µg/g, respectively. Sediments containing more than 89 µg/g of PAHs suppress at least half of the photoactivity of *Photobacterium phosphoreum.* The *Photobacterium* bioassay responds mainly to sulphide toxicity, and sulphides block biodegradation of PAHs. Thus, the most acutely toxic sediments will maintain the potential to induce tumours for a long time. The mean "LC_{50}" of these three bioassays, 200 µg/g is suggested as an interim cleanup guideline. In one area of the harbour near Randle Reef, the total concentration of PAHs in the 48,300 m^3 of near-surface sediments exceeds 200 µg/g. The consequences of using biological standards is discussed, i.e., is dredging required for the most contaminated sediments, and is in-situ bioremediation adequate for moderately contaminated sediments?

ECOTOXICOLOGICAL HAZARD ASSESSMENT OF HYDROCARBON CONTAMINATED SOILS: A CASE STUDY.

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The Ecotoxicological Hazard Assessment (EHA) developed by the Québec Ministry of Environment and Wildlife was used as part of the management scheme of contaminated soils from a former refinery. The study consists of assessing five types of soils (reference, heavily contaminated, slightly contaminated, thermally-treated, and biotreated) to determine their relative intrinsic hazard. During the exploratory activities a series of ten assessment endpoints where identified to support this typical EHA. During SOURCE characterization, the physico-chemical make-up of the soils is described and the presence and concentrations of priority pollutants is determined. During FATE characterization, the potential for bioconcentration, mobility, and persistence of pollutants is determined. During EFFECTS characterization, the soils and their leachates are tested using standard terrestrial and aquatic bioassays. The data from the toxicological and analytical testing program are evaluated semi-quantitatively on the basis of a scoring system developed by consensus. The discussion will highlight how data are used within an EHA to streamline the decision-making process regarding the follow-up cleanup and disposal of contaminated soils.

SOIL ECOTOXICOLOGICAL TESTING FOR CANADIAN SOIL QUALITY GUIDELINES.

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Soil ecotoxicological testing is being used to support the development of soil quality criteria. Environment Canada sponsored five ecotoxicity tests for 20 priority substances. The test included root elongation and seed germination for radish and lettuce, as well as the 14 day survival test for the earthworm *Eisenia foetida*. Modifications were made to the test protocols of Greene et. al. (1989) for testing of VOC's (volatile organic compounds). The results of these tests represent one of the most comprehensive soil toxicity datasets available. Recommendations for further testing requirements and directions will be provided.

TOXICITY TESTS ON SOILS AND SOIL EXTRACTS - TOWARDS A SOLID PHASE TIE.

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Contaminated site assessment and remediation is conventionally based on chemical-specific criteria. Howöever, the parameters that render a soil toxic may not be included in the list of targeted or priority substances. It is essential to identify the toxic substances in order to fully assess the risk and develop viable remediation strategies. A procedure was developed for fractionating soils to isolate and characterize the toxic constituents (solid phase toxicity identification evaluation or TIE). The soil is sequentially extracted with different solvent systems. Toxicity reduction is measured after each extraction step. The extracts are fractionated at acidic, neutral, and basic conditions. Insoluble materials are removed by filtration. Organics are removed by solid phase extraction and subsequent elution with solutions of methanol in water. The toxic fractions are added back to extracted soils in the final confirmation phase. The method is presented along with results from solid phase TIE's conducted on contaminated soils from a sour gas plant in Western Canada. This method is a useful tool for isolating and identifying the toxic constituents present in contaminated soils.

CANADIAN SOIL QUALITY CRITERIA. C. Gaudet, P. Cureton, D. Milne, S. Teed. Guidelines Division Environment Canada Environment Canada, Ottawa, ON, K1A 0H3.

As part of the CCME National Contaminated Site Remediation Program (NCSRP), Environment Canada, Health Canada and the Provinces and Territories have been working collaboratively to develop national guidance for contaminated site assessment. This guidance is based on a set of national "scientific tools" which support an integrated, tiered framework for contaminated site assessment and remediation in Canada. A major emphasis of this program is the development of national soil quality criteria for the protection of agricultural, residential/parkland and commercial/industrial land uses in Canada. A national protocol for the development of Canadian soil quality criteria has been developed and assessments and draft criteria (ecological-effects) have been finalized for 15 priority contaminants in Canadian environment. During these assessments, it was apparent that there is a general lack of ecotoxicological data for soil organisms suitable for the derivation of soil quality criteria. To overcome this on the short term, Environment Canada has limited toxicological testing conducted for 15 priority contaminants. However, there is an urgent need for an increased emphasis on soil ecotoxicology if we are to deal effectively with the management of contaminated lands.

was developed. Most of these compounds work as mimics of the key steroid hormone 17ß-estradiol (E_2) by interacting with the receptor. We isolated E_2 receptors from immature E_2 primed rainbow trout (*Oncorhynchus mykiss*) liver, a target organ for E_2. E_2 binding activity was estimated by using a radioassay using [^{3}H]E_2. The receptors identified were highly specific, saturable and exhibited high affinity and low capacity. A competition assay was developed where a constant amount of [^{3}H]E_2 was incubated with increasing concentrations of a competitor. Known estrogenic compounds including diethylstillbestrol (DES) and genistein (a phytoestrogen) were shown to compete with E_2 for the receptors. We tested detergent samples (nonylphenols) found in treated industrial and municipal sewage waters as well as in pulp and paper mill effluents. Using our system, we found that some nonylphenols have estrogenic activity at concentrations relevant to what has been found in nature.

GENOTOXICITY TESTING, APPLICATIONS IN THE AQUATIC ENVIRONMENT. SESSION CHAIR: C.D. METCALFE.

DEVELOPMENT OF A HEPATIC MICRONUCLEUS ASSAY IN FISH. C.D. Metcalfe and C.R. Williams. Environmental and Resource Studies Program, Trent University, Peterborough, ON, K9J 7B8.

An *in vivo* hepatic micronucleus assay with rainbow trout was developed to test for the genotoxic effects of aquatic contaminants. An assay protocol was developed in which proliferation of hepatocytes in the trout liver is stimulated by a regenerative response after exposure to allyl formate (AF). The indirect-acting clastogen, diethylnitrosamine (DEN), and the direct-acting clastogens, ethyl methanesulphonate and mitomycin C, all gave a positive response in this assay. The assay was tested for potential as a biomarker of genotoxicity in fish by sampling English sole from Puget Sound, Washington. Sole from the contaminated Duwamish Estuary had an elevated micronucleus frequency of 5 micronuclei per 1000 hepatocytes in comparison to a mean micronucleus frequency of 0.5 micronuclei per 1000 hepatocytes in sole from a relatively pristine area of Puget Sound.

MONITORING GENETIC DAMAGE IN TWO SPECIES OF FISH. R. Pandrangi, S. Ralph, K. Perry, M. Vrzoc and M. Petras. Department of Biological Sciences, University of Windsor, Windsor, ON, N9B 3P4.

Monitoring genotoxicity of the environment using endemic organisms as sentinels requires the development of sensitive assays. Since standard assays were not suitable in our target organisms, we explored the possibility of applying the SINGLE CELL DNA ELECTROPHORESIS (SCDE) technique (Singh et al.: Expt. Cell Res. 175:184). This approach involves detection of DNA fragments which on electrophoresis migrate from the nuclear mass resulting in a 'comet with tail' formation. Tail length has been correlated with the level of genotoxicant exposure. The fish used in the present study were brown bullheads (*Ameriurus nebulosus*) and carp (*Cyprinus carpio*). On electrophoresis of erythrocytic DNA we found a linear increase in the tail length / width ratio over a broad range of doses of cyclophosphamide and methyl methanesulfonate. Freshly caught bullheads from six different sites showed a wide range of DNA damage. Bullheads from Big Creek, Hamilton Harbour and the Detroit River gave ratios of 3.3 to 4.5. Bullheads from southern part of Lake Huron and from a fish hatchery in Brockport, New York gave ratios around 1.3. Bullheads from Big Creek, maintained in the laboratory for three months gave ratios which approached those from the hatchery (1.4). Results for carp were similar to the above. Carp from Big Creek gave ratios about 4.5, while carp from Lake Huron and laboratory maintained carp gave values between 1.2 and 1.4. The results from the SCDE assay in bullheads and carp indicate that the SCDE assay is extremely sensitive and should be useful in detecting DNA damage caused by environmental contaminants. (Supported by Great Lakes University Research Fund.)

EVALUATION OF GENOTOXIC POTENTIAL OF BLEACHED KRAFT MILL EFFLUENT USING BACTERIAL AND MAMMALIAN CELLS.

S.S. Rao[1], M.A. Hayes[2], B.K. Burnison[1], D.A. Rokosh[3] and P.D. Hansen[4]. [1]National Water Research Institute, Canada Centre for Inland Waters, Burlington, ON, L7R 4A6; [2]Department of Pathology, University of Guelph, Guelph, ON, N1G 2W1; [3]Biohazard Laboratory, Ministry of Environment and Energy, Rexdale, ON, M9P 3V6; [4] Institute of Ecology, Technical University of Berlin, Goerzalle 253, 14167, Germany.

Bleached kraft mill effluent (BKME) has been shown to contain mutagenic activity and induce certain physiological disturbances in fish such as increased activity of MFO enzyme system. Recent work has shown that the Ames mutagenic substances in BKME were direct acting mutagens, and that the majority of these substances were recoverable in XAD-4 and XAD-8 resins using NaOH and methanol elution technique. Mutagenic materials are considered genotoxic, posing a risk factor for genetic damage, teratogenicity, embriotoxicity, genetic disease and carcinogenesis. This study investigates the mutagenic potency of Bleached kraft mill effluent, and assesses the genotoxic potential (DNA damaging effects) of certain mutagenic fractions in both bacterial and mammalian cells. While mutagenic potency of effluent was determined using the Ames fluctuation assay, the DNA damaging potential was measured using the *Umu-C* bacterial genotoxicity assay and mammalian cell susceptibility assay using Chinese hamster lung V79-4 cells. Data indicate that the majority of the mutagenic substances were effectively absorbed to XAD resins and recovered in methanol and NaOH and the responses of test cells to these mutagenic substances were comparable, suggesting that the test protocols were complementary, practical and applicable in the screening of environmental samples for assessing their genotoxic potential. Data and some discussions on the usefulness of these protocols are presented.

COMPARING THE PRESENCE, POTENCY AND PARTITIONING BEHAVIOUR OF GENOTOXINS EXTRACTED FROM A BROAD RANGE OF INDUSTRIAL EFFLUENTS.

P.A. White[1], J.B. Rasmussen[1] and C. Blaise[2]. [1]McGill University, Department of Biology, 1205 Dr. Penfield Avenue, Montreal, QC, H3A 1B1; [2]The St. Lawrence Center, Environment Canada, 1001 Pierre Dupuy Avenue, Longueuil, QC, J4K 1A1.

We examined the genotoxicity of dichloromethane extracts from the final effluents of 42 industries- including pulp and paper, chemical manufacturing, metal refining, metal surface treatment and municipal wastewater treatment. Effluents were initially fractionated into dissolved substances and substances adsorbed to suspended particulate matter. Acid/base partitioning was used to further fractionate aqueous extracts. Genotoxicity was measured on the SOS Chromotest. Potency and contamination of extracts was related to sample type, industry type, and the concentration of polycyclic aromatic hydrocarbons. S9 metabolic activation reduced genotoxic potency in over 90% of the extracts examined. SOS genotoxic potency values correspond well with Ames mutagenic potency values described by other researchers. Sorption partition coefficients were calculated to quantify the affinity of industrial genotoxins for suspended particulate matter. Partition coefficients ranged from 10^3 for pulp and paper mill samples to almost 10^7 for aluminum refinery samples. With the exception of pulp and paper mills and sewage treatment facilities, over

50% of the emitted SOS genotoxins are adsorbed to effluent suspended particulate matter. Genotoxic loading values were calculated to quantify the total daily genotoxic emission of each industry. Highest loadings are from sewage treatment, pulp and paper and metal refining facilities. The ultimate hazard of the genotoxic emissions is not known. Hazard assessment is complicated by a poor understanding of the post-emission behavior of genotoxins. Exposure of downstream biota is likely substantial.

DETERMINATION AND GENOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS ISOLATED FROM *Dreissina polymorpha* (ZEBRA MUSSELS) SAMPLED FROM HAMILTON HARBOUR. C.H. Marvin[1] and B.E. McCarry[1] and D.W. Bryant[2]. [1]Department of Chemistry, McMaster University, Hamilton, ON, L8S 4M1; [2]Department of Biochemistry, McMaster University, Hamilton, Ontario L8N 3Z5.

Polycyclic aromatic hydrocarbons (PAH) were determined in extracts of zebra mussels sampled from sites in Hamilton Harbour. The mussels were extracted using a tissue homogenizer method and the resulting solvent extracts were subjected to an alumina column and a Sephadex LH20 column clean-up procedure. Total PAH concentrations in Zebra mussel extracts ranged from 0.35 µg/g to 10.0 µg/g of mussel homogenate. The extracts were also found to exhibit significant mutagenic responses in the Ames *Salmonella*/microsome assay. In contrast to the suspended sediments, the mutagens in zebra mussel tissue are primarily of the type which require microsomal activation. The results indicate that Zebra mussels might be used as biological indicators of PAH contamination in fresh water environments such as Hamilton Harbour.

EXPOSURE-EFFECTS OF DETROIT RIVER SEDIMENTS ON BROWN BULLHEADS (*Amerious nebulosus*). T.A. Leadley, R. Lazar and G.D. Haffner. Great Lakes Institute for Environmental Sciences and Department of Biology, University of Windsor, Windsor, ON, N9B 3P4.

In order to investigate the exposure-effects of contaminants on a benthic feeding fish in the Detroit River, sediments and Brown bullhead catfish (*Amerious nebulosus)* were sampled from three distinct areas within the river, Peche Island, Trenton channel, and the Amerstburg channel. Both sediments and bullhead muscsle tissue were analyzed for various OCC's and PAH's. A histophathology assessment of the bullhead livers was also conducted. Initial findings indicate that both the sediment contaminant load and the bullhead body burdens were higher in the Trenton channel samples. Results from the liver histological assessment revealed a higher incidence of both pre-malignant and malignant lesions in the Trenton channel bullheads.

LIVER NEOPLASIA IN WHITE SUCKERS FROM LAKE SUPERIOR, LAKE ONTARIO

AND THE ST. LAWRENCE RIVER. I.R. Smith[1], C.B. Portt[2], M.A. Hayes[3] and D.A. Rokosh[1]. [1]Ministry of the Environment and Energy, 40 St. Clair Ave. W., Toronto, ON, M4V 1M2; [2]Portt and Associates, 56 Waterloo St., Guelph, ON, NIH 3H5; [3]Department of Pathology, University of Guelph, Guelph, ON, N1G 2W1.

The incidence of liver neoplasia, as an indicator of exposure to carcinogenic contaminants, has been investigated (between 1985 and 1990) in white suckers, *Catostomus commersoni*, from several Great Lakes Areas of Concern (AOC). Liver neoplasms were found in 5.8, 6.1, 7.1, 7.1, 8.1 and 9.2 % of the white suckers (n=1093) examined from five AOCs and one nearby site dominated by industrial and municipal discharges, but no neoplasms were found in suckers (n=91) from a fifth AOC dominated by agricultural and municipal discharges. More than 71% of these neoplasms were derived from bile duct epithelium, the remainder originating from hepatocytes. The incidence of liver neoplasia was low (0.0, 0.0, 0.0, 0.4 and 2.4%) in suckers (n=572) from five reference locations. A sample of suckers (n=230) from an additional reference location, Batchewana Bay on Lake Superior (believed to be contaminated only by atmospheric deposition and lampricide application), featured a 23% incidence of cancer in suckers older than 15 and an overall incidence of 8.6%, suggesting that neoplasms can develop in the apparent absence of localized chemical inputs. The relation between liver neoplasia and inflammation, parasitic infections, bile duct proliferation, and hepatic MFO induction, suggests neoplasms may be a response to the presence of synthetic chemicals and liver parasites as co-carcinogens.

PLANT TOXICOLOGY
SESSION CHAIR: B.A. HALE

QSAR MODEL FOR THE PHOTOINDUCED TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO AQUATIC PLANTS.

B.M. Greenberg, S.N. Krylov, X.-D. Huang and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

A model for photoinduced toxicity of PAHs to duckweed (*Lemna gibba*) was developed. Toxicity was measured as suppression (or inhibition) of growth. Growth suppression was described by photochemical reactions between PAHs and a hypothetical group of biomolecules (given the notation **G**) which are postulated to be required for normal growth of the plants. Light activation of PAHs was considered in a two compartment system (water column and leaf tissue). The reaction scheme includes: photooxidation of PAHs, partitioning of PAHs into leaves, triplet-state formation of intact PAHs, photosensitization reactions that consume **G**, and reactions between photooxidized PAHs and **G**. The assumptions used in the model are: the rate of PAH photooxidation is slower than the rate of assimilation, PAH content in solution is approximately constant over the length of the toxicity test, the fluence rate of actinic radiation is lower in the leaves than in solution, the growth suppression caused by intact PAHs in the absence of light is negligible, and the reaction of photooxidized PAHs with **G** does not require light. We then derived and solved a series of differential equations that described plant growth in the presence of PAHs and simulated solar radiation (SSR). The resultant expression for suppression of growth was a function of the treatment period, the initial concentration of the PAH, the integral of the overlap between the output spectrum of SSR source and the absorption spectrum of the PAH in question, the quantum yield for formation of triplet state PAH and the rate of photooxidation of the PAH. The expression also included two complex constants: the first described the toxicity of photoproducts and the second described the photosensitization processes initiated by PAHs (i.e. reactions of activated O_2 with **G**). For each chemical we estimated the first constant by a least squares analysis of the empirical growth data assuming that the second constant is invariant for each PAH. The model accurately describes photoinduced toxicity based on a linear relationship between growth suppression and the above photochemical parameters ($r^2 > 0.9$).

PHOTOINDUCED TOXICITY AND ASSIMILATION OF SEDIMENT BOUND POLYCYCLIC AROMATIC HYDROCARBONS TO THE AQUATIC PLANT *Lemna gibba*.

B.M. Greenberg, C.L. Duxbury, C.A. Marwood and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

Light, in particular UV irradiation, is known to increase the toxicity of polycyclic aromatic hydrocarbons (PAHs). Since aquatic plants cannot avoid PAHs or light, they are at risk. However, the major loadings of PAHs are in sediment. In this study we examined the uptake of both intact and photomodified ^{14}C-labelled PAHs bound to a surrogate sediment (sand) by the higher aquatic plant *Lemna gibba* L. G-3. It was found that simulated solar radiation (SSR) significantly promoted the release of PAHs from sand and their assimilation by *L. gibba*. Although assimilation from the solid phase was slower than for chemicals placed directly into the aqueous medium, it was fast enough to contribute to toxicity. Therefore, we examined the photoinduced toxicity of PAHs bound to sand by exposing *L. gibba* to the sand-chemical composite for 8 d. Plants were exposed to both visible and SSR light conditions during this period, and toxicity was measured as inhibition of growth, inhibition of photosynthesis (chlorophyll fluorescence induction) and chlorosis. All three end-points indicated that PAHs bound to a sediment phase can express phototoxicity and that the effects are most strongly promoted by the UV-region of the solar spectrum. We extended this work to show that PAHs released from sand in photomodified form are toxic to *L. gibba*. This is similar to our previous findings that showed if PAHs are added directly to the medium and photomodified in solution they are highly toxic. Additionally, we probed the effects of UV-B and PAHs on chloroplast development, with the finding that both stresses inhibit development of the photosynthetic apparatus.

BIOTRANSFORMATION OF MERCURY BY PROKARYOTIC AND EUKARYOTIC FRESHWATER ALGAE IN PH-STAT CULTURES. D.J.A. Kelly[1], G.W. Van Loon[2], K. Budd[1] and D.D. Lefebvre[1]. [1]Department of Biology, and [2]Department of Chemistry, Queen's University, Kingston, ON, K7L 3N6.

Investigations were conducted into the biotransformation of subinhibitory and growth inhibitory levels of $HgCl_2$, by isolates of cyanobacteria and green algea. Cultures were grown in pH-stat batch cultures. The observation was made that the contribution of volatilization and uptake by the cells varied with the organism. It was also observed that the initial $HgCl_2$ dose can be fully detected in whole cultures by reduction under acid conditions with $SnCl_2$. However, shortly after the introduction of mercury, it is converted to a form insensitive to reduction under acid conditions, but fully detectable under alkaline conditions. Following a finding by a chemistry research group studying sulfhydryl compounds, this difference in assay for mercury was applied to whole cell cultures. However, our evidence is consistent with the identity of the algal mercurial being HgS. Phytoplankton tows have confirmed the presence of this form in material collected in various locations. This chemical method has several advantages over digestion techniques and can be conducted in real time with minimum sample preparation. We report results from the study of treatments which alter the balance between fixation as HgS and evolution of mercury as HgO. These have the potential to affect the residence time of mercury in a watershed, and flux to the atmosphere.

EFFET D'UN AGENT COMPLEXANT SUR LA CINÉTIQUE D'ACCUMULATION ET D'ÉLINATION DU CADMIUM CHEZ DEUX BRYOPHYTES: *Fontinalis dalecarlica* ET *Platyhypnidium ripariroïs*. G. Samson, G. Vaillancourt et L. Pazdernik. Université du Québec à Trois-Rivières, Département de chimie-biologie, C.P. 500, Trois-Rivières, QC, G9A 5H7.

Un des principaux inconvénients rencontré lors de l'analyse des métaux lourds résulte des

faibles concentrations présentes dans le milieu aquatique. Dans ces cas, l'utilisation de bioindicateurs est souvent nécessaire pour suivre l'évolution spatio-temporelle des polluants. Les mousses aquatiques choisies sont des biomoniteurs de substances toxiques capables d'informer sur l'état de l'environnement(Berryman et Richards, 1992). Dans le passé, plusieurs méthodes ont été développées pour mieux comprendre ces interactions biologiques; toutefois, maintenir la concentration voulue sans variations importantes a toujours constituée une difficulté majeure. Le système expérimental mis au point dans cette étude est simple; il privilégie l'écoulement gravitationnel plutôt que mécanique. Un contrôle manuel permet de maintenir le débit constant dans ce système dynamique. L'expérimentation a été effectuée aux concentrations de 10, 25 et 50 µg/L avec et sans agent complexant "EDTA". L'objectif visé dans ce travail est d'évaluer la performance de deux mousses aquatiques *Fontinalis dalecarlica* Schimp. ex B.S.G. et *Platyhypnidium riparioïdes* (Hedw) Dix. lors de l'accumulation et de l'élimination du cadmium pendant 28 jours pour chacun des cycles. Les résultats prélimilaires indiquent une bioaccumulation élevée pendant les premiers jours. En absence de l'agent complexant, les concentrations en cadmium varient entre 150 et 300 µg/g selon l'espèce. A la suite des résultats obtenus, il semble que les mousses subissent un débalancement ioniques internes lorsque la période d'accumulation est trop longue (28 jours). Dans ce cas, l'élimination est lente et le relargage du cadmium est peu perceptible après 1 mois (stress physiologique). L'utilisation d'EDTA est rendu nécessaire pour 1) maintenir constante les concentrations du polluant, 2) diminuer la biodisponibilité du métal étudié, 3) se rapprocher des conditions naturelles du milieu.

BIOMARKERS
SESSION CO-CHAIRS: P.G.C. CAMPBELL AND C.G. ROUSSEAUX

ENVIRONMENTAL BIOMARKERS: FICTION, FANTASY, OR FUNCTIONAL? L.S. McCarty[1] and K.R. Munkittrick[2]. [1]L.S. McCarty Scientific Research & Consulting, 280 Glen Oak Drive, Oakville, ON, L6K 2J2; [2]GLLFAS, Department of Fisheries and Oceans, Burlington, ON, L7R 4A6.

The first biomarkers used in ecotoxicology were biochemical in nature. Subsequently, this approach was extended to other levels of biological organization. Although popular biomarkers can be problematic. They can be viewed as a "short-cut" methodology which must ultimately be based on a clear understanding of an established biological indicator. Thus, a biomarker should be a quantitative or semi-quantitative surrogate measurement for either cause or effect (i.e., dose or response). If the biomarker-biological indicator relationship is established a biomarker can be a useful, practical, scientifically valid tool. Unfortunately, validation is not common and some current biochemical biomarker approaches are critically examined in this regard under the categories of exposure (dose), effect (response), and causality. Observations include (1) most popular biochemical biomarkers provide only qualitative estimates of exposure which are of underwhelming value as some exposure can essentially be assumed at many sites of concern; (2) at the same site different biomarkers can suggest exactly opposite trends and conclusions, and (3) correlation between a biomarker and an effect does NOT prove causality.

Biomarkers, biochemical or otherwise, represent a "short-cut" that should be used only after both a general validation of the concept and a site-specific confirmation of applicability. To do otherwise is an affront to scientific methodology and a waste of increasingly scarce scientific funding. The biomarker approach, originally developed in pharmacology/medical toxicology, has become popular for use in environmental toxicology and monitoring. Despite this popularity questions about relevance remain. Irrespective of "top-down" or "bottom-up" investigative approaches, a basic assumption of toxicology - cause-effect, usually expressed as dose-response - must always be addressed. Specifically, to be of practical use and scientifically valid, a biomarker must be a quantitative or semi-quantitative surrogate for either dose or response i.e., it must correspond to or represent a valid biological indicator. As this requirement is often neglected in environmental studies several problems occur. (1) As qualitative indications of occurrence of exposure biomarkers are of underwhelming significance as exposure at some level can be often be assumed. (2) Biomarkers workers often assume that cause-effect linkages are established without any validation. (3) For many poorly-metabolized organic chemicals the concentration of the chemical in the body of an organism is likely a better and less ambiguous measurement/surrogate for dose than biochemical biomarkers. (4) Biomarkers can be used as a "short-cut" only after both a general validation and a site-specific confirmation, not before. Current and potential uses of biomarkers for various classes of environmental indicators will be reviewed with reference to requirements for validity and utility . Classes of Biological Indicators: [1] biochemical (enzymes, hormones); [2] physiological (growth, reproduction, nutrition), [3] toxicological (behaviour, lethality, teratogenicity, mutagenicity, carcinogenicity); and [4] ecological/community (additions, deletions, alterations in ecosystem/community structures and relationships).

BIOMARKERS FOR AQUATIC ECOSYSTEM HEALTH - THE ETIOLOGY OF EPIDERMAL PAPILLOMA IN WHITE SUCKER.

P.D. Premdas[1,2] and C.D. Metcalfe[2]. [1]Department of Biology, Queen's University, Kingston, ON, K7L 3N8; [2]Environmental and Resource Studies Program, Trent University, Peterborough, ON, K9J 7B8.

There is a need to develop "biomarkers" for assessing the health of aquatic ecosystems. The white sucker, *Catostomus commersoni*, has been proposed as a sentinel species to monitor environmental health in the Great Lakes. Visible neoplasms may provide identifiable "biomarkers" to reflect changes in ecosystem health. Papilloma prevalence in white suckers is elevated in contaminated areas, but these lesions are also relatively common in uncontaminated regions. This project is directed at determining the etiology of epidermal papillomas in white suckers. External neoplasms can be differentiated from non-neoplastic tissue on the basis of biochemical (Protein Kinase C) activity. Our findings indicate that many instances of "spontaneous" regression or development of papilloma in captive suckers are the result of culture conditions. The size and growth rates of epidermal papilloma on white suckers can be regulated by testosterone and ethinyl estradiol. Exposure to these steroid hormones increased the size and maintained the frequency of papilloma in captive suckers. Tumors were successfully transmitted into hatchery-raised suckers by injecting them with cell-free filtrates prepared from lip papillomas. These results give some indication of the primary etiological agents responsible for the development of papillomas in white suckers. It is anticipated that this study will establish whether the induction of white sucker papillomas occurs as a result of viral infection, exposure to chemicals, or changes in hormonal status, or by interactions between all of these factors.

BIOMARKERS/METALLOTHIONEIN
SESSION CO-CHAIRS: P.G.C. CAMPBELL AND C.G. ROUSEAUX

METALLOTHIONEIN: FUNCTION IN CELLULAR REGULATION AND USE AS A BIOMARKER. D.W. Engel. NOAA/National Marine Fisheries Service, Southeast Fisheries Science Center, Beaufort Laboratory, Beaufort, NC.

For years the primary role of metallothionein (MT) has been viewed as a low molecular weight metal-binding protein that is synthesized in prokaryotic and eukaryotic organisms as a response to exposure to contaminant transition metals. Now, however, this view of MT has broadened to include its involvement in reactions to stress, and in normal metabolism and trace metal partitioning in both vertebrates and invertebrates. Some of the best examples of MT's involvement in constitutive metal regulation come from research on the molting processes in marine crustaceans. In addition, it has been suggested that MT may play an important role in the sequestration of free radicals generated through normal metabolic processes as well as through the metabolism/detoxification of xenobiotics. The use of MT as a biomarker for metals may be viable, but changes in concentrations or induction of MTs in aquatic organisms must be considered in light of the current information on MT metabolism.

INDUCTION OF METALLOTHIONEIN GENE EXPRESSION BY EPIDERMAL GROWTH FACTOR IN RAT HEPATOCYTES. F. Denizeau, P. Moffatt, M. Lachapelle and G.L. Plaa. CIRTOX, Université de Montréal, C.P. 6128, Succ. Centre–Ville, Montréal, QC, H3C 3J7; Université du Québec à Montréal, C.P. 8888, Succ. Centre–Ville, Montréal, QC, H3C 3P8.

Metallothionein (MT) is a small cysteine–rich protein thought to be mainly involved in metal regulation and detoxification. The implication of MT in cell growth and differentiation has also been suggested. This latter hypothesis was further investigated in adult rat hepatocytes induced to proliferate by epidermal growth factor (EGF). Exposure of hepatocytes to EGF resulted in significant increases in MT protein and MT–1 mRNA levels. As revealed by nuclear run–on analysis, these changes were the result of transcriptional activation. Addition of $ZnSO_4$ or dexamethasone (Dex) were also effective at inducing MT protein and mRNA. Combined addition of Zn and EGF produced an additive increase in MT protein and MT–1 mRNA levels. When both Dex and EGF were present together, the EGF–induced MT protein and mRNA expression was lost whereas it had only minor inhibitory effects on DNA synthesis. TGF–_, a known antagonist of EGF on hepatocytes, blocked the EGF–induced MT accumulation. MT immunoreactive sites were visualized using protein A–gold labeling and electron microscopy. In Zn–exposed hepatocytes (24h), the labeling density was increased in the cytosol. Whereas EGF treatment alone did not cause any changes in MT distribution at that time, the Zn+EGF combination produced an equally important cytosolic and nuclear staining, to a level that was approximately twice that found in control cells. In addition, the EGF–induced *c–fos* mRNA accumulation was blocked by Dex whereas TGF–_ had no effect. These results show that growth factors believed to play a role in liver regeneraiton can modulate MT gene expression. The possibility that *c–fos* stimulation may play a role in MT induction by EGF cannot be ruled out. This work was supported by NSERC and CNTC.

COMPETITIVE INTERACTIONS BETWEEN Cd AND Cu FOR METALLOTHIONEIN-LIKE PROTEINS. A.Z. Mason. Department of Biological Sciences, California State University Long Beach, CA, 90840.

The effects of Cd intoxication on the accumulation and cytosolic distribution of Cu have been studied in the marine polychaete *Neanthes arenaceodentata* and intertidal mollusc *Littorina littorea.* Animals were exposed to EDTA metal-chelate buffered seawater containing either low ($10^{-10.2}$ - $10^{-11.7}$ M) or elevated concentrations of Cd ($10^{-6.1}$ - 10^{-7} M). The free Cu ion activity was identical for both the control and Cd exposed groups of animals ($10^{-11.4}$ for polychaetes; 10^{-12} M for the molluscs). Two major observations were noted in both species in response to Cd exposure. First, enhanced accumulation of cytosolic Cu was noted. Experiments involving radioisotopic ^{64}Cu and stable isotopic ^{65}Cu indicated that a large component of the accumulated metal was scavenged intrinsically, presumably from non-cytosolic stores of the metal. Only a small component of the accumulated Cu was derived extrinsically. Second, HPLC/ICP-MS analyses showed major changes in the cytosolic distribution of Cu upon Cd exposure. This redistribution was attributed to the induction of metallothionein like proteins by Cd and the subsequent sequestration of Cu from Cu containing non-cytosolic and cytosolic ligands by these newly synthesized proteins. Presumably this sequestration results from the preferential binding of MT for Cu>Cd. This data is toxicologically provocative since it indicates that Cd may, in certain instances, exert an indirect toxicological effect by causing the depletion of Cu from cellular enzymes that require its presence allosterically or catalytically for normal functioning. Paradoxically, if this interpretation is true, then the detrimental effects induced by Cd exposure may be ameliorated in environments containing elevated concentrations of Cu.

HEPATIC METALLOTHIONEIN CONCENTRATIONS IN WHITE SUCKER (CATOSTOMUS COMMERSONI) FROM THE ST. LAWRENCE RIVER: 1. EFFECTS OF STORAGE CONDITIONS; 2. CORRELATION WITH HEPATIC HEAVY METALS. H.H. Sloterdijk, A.-M. Prud'homme and J. Bureau. Centre Saint-Laurent, Environment Canada, 105 rue McGill, Montreal, QC, H2Y 2E7.

ABSTRACT

White sucker (*Catostomus commersoni*), a benthic fish species, were captured by electrofishing in the St. Lawrence River near Montreal, Quebec, and livers were dissected out for metallothionein (MT) determination (silver-saturation method). The stability of hepatic metallothionein (MT) was investigated in order to develop standardized freezing and storage procedures for liver samples: stored at -145°C, either as S10 fraction, homogenates, or pieces of liver for periods varying between 7 and 168 days. MT levels were maintained more efficient in the stored S10 fractions than in either the stored

homogenates or liver pieces. Storage period seemed to cause some decrease in MT levels, although the difference was significant only for the 168 days storage. Exposure experiments *in situ* were carried out in August, 1991. Feral fish were put into cages placed for about a week upstream and downstream of an industrial complex (heavy metals). MT and hepatic metal content was determined. A direct relationship between hepatic copper and zinc concentrations and MT content was observed, with concentrations being higher in specimens from the upstream site. Recent data on sediments from the same sites suggest that MT induction in white sucker may be more related to sediment contamination (Cu and Zn) than to the presence of point sources.

INTRODUCTION

Biochemical indicators of water quality have been increasingly used over the last few decades. Among these, complexing proteins such as metallothioneins (MT), a polypeptide of about 6000-7000 DA, have been investigated in several fish populations (Roch et al. 1982, McCarter and Roch 1983, Klaverkamp et al. 1984, 1991). Its role is thought to be the metabolic regulation of the essential metals Cu and Zn, and the detoxification of the non-essential metals Cd and Hg (Roesijadi 1992).

To explore its potential as a biomarker, a study on hepatic metallothionein in feral white sucker, a common benthic species in the St. Lawrence River, was carried out in 1991. Specimens were taken in Lake St. Louis near Beauharnois (Fig. 1). This region is known for its industrial complexes, discharging metals such as Cu, Zn, Cd, and Hg to the St. Lawrence River.

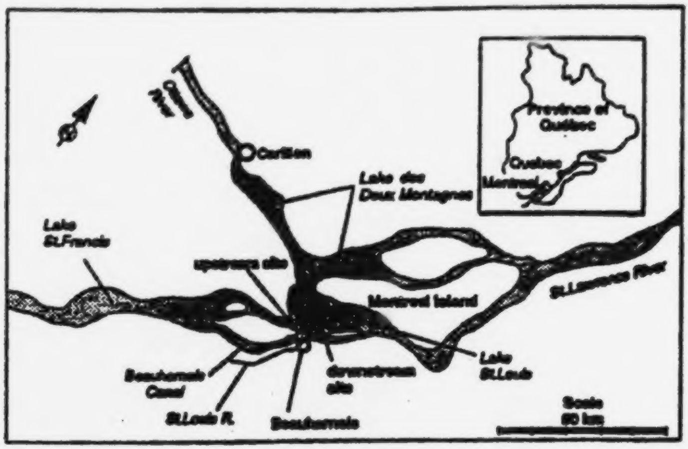
Carillon
Lake des Deux Montagnes
Province of Québec
Quebec
Montreal
Lake St.Francis
upstream site
Montreal Island
St.Lawrence River
Beauharnois Canal
St.Louis R.
Beauharnois
downstream site
Lake St.Louis
Scale
50 km

FIG. 1. St. Lawrence River and the study area.

The stability of hepatic metallothionein (MT) was investigated in order to develop standardized freezing and storage procedures for liver samples (Prud'homme et al. 1993). *In situ* exposure experiments were also carried out, where feral fish were put into cages placed upstream and downstream of an industrial complex. The study design was based, among others, on the working hypothesis stating that fish living downstream would show higher MT levels than those living upstream of the industrial complex. Although three experiments were carried, only one (the 2nd experiment), for which results are presented here, included hepatic heavy metal analyses (Sloterdijk et al. 1993).

MATERIALS AND METHODS

Feral white sucker (*Catostomus commersoni* Lacepede) were captured by electrofishing in the vicinity of the industrial complex at Beauharnois, Lake St. Louis (St. Lawrence River, Fig. 1). On site, fish were stunned by a blow on the head and livers were dissected out immediately, divided into subsamples for MT and heavy metal analysis, and conserved in liquid nitrogen. After arrival in the laboratory, the samples were kept in a cryofreezer at -145°C. At analysis, the MT liver subsamples were thawed out and homogenized in cold 250 mM sucrose buffer of pH 7.4, containing 20% glycerol, followed by centrifugation at 10 000 X g for 30 min at 4°C, yielding the supernatant, the S10 fraction (also known as the postmitochondrial fraction). MT concentrations in the S10 fraction were determined in duplicate using the Ag-saturation method (Scheuhammer and Cherian 1986), which was modified for use in our laboratory by Gagné (1991). MT concentrations are expressed as pmoles or nmoles/mg protein, the latter having been determined in the S10 fraction by a colorimetric method (Bradford 1976). Heavy metals (Cu, Zn, Cd, Hg) were analysed in subsamples of liver according to standard methods developed by Environment Canada (1979) and Best (1981). Metal results are expressed as µg/g wet weight.

Preservation study: Twenty fish were collected, whose livers were dissected out immediately on site, cut into small pieces, and pooled two by two, resulting in 10 samples. Each of these 10 samples was then divided into three groups (A, B and C) for the following treatments: A and B were homogenized with an Omni 1000 cordless homogenizer and frozen in liquid nitrogen; subsamples C were frozen directly in liquid nitrogen. Homogenates A were thawed out the following day in the laboratory and centrifuged. The resulting supernatant or S10 fraction (A), as well as the liver homogenates (B) and cut liver pieces (C) were stored at -145°C until analysis. Storage time until MT analysis of treatment groups A, B, and C (each of which had been split into 6 subsamples) ranged from 2, 7, 14, 21, 84, and 168 days, referred to as t1, t2, t3, t4, t5, and t6, respectively. At the time of the MT analysis, the S10 fractions were prepared from the liver homogenates (treatment B) and the liver pieces (treatment C).

Caged fish study: The fish were caught by electrofishing at two sites, one upstream (u) and at the other downstream (d) of the industrial complex at Beauharnois (Fig 1). Temperature, conductivity, alkalinity and hardness of the ambient waters were around 23°C, 300 µS/cm, 90 mg/L and 125 mg/L (the latter two as $CaCO_3$ equivalents), respectively, at both sites. Oxygen levels were near or at saturation values. The fish were set out in 6 cages (about 8-10 individuals per cage) on the bottom at each site (Z = 5-7 m), where 3 cages contained fish from the same site (i.e. without transfer), while the 3 other cages contained fish from the other site (i.e. with transfer). The 6 m long cylindrical fyke-net-style cages consisted of three metallic hoops, 1.5 m in diameter, covered with a 25 mm mesh net and held together with three metallic bars (6.5 mm ϕ).

The exposure experiment was carried out at the beginning of August, 1991, and lasted 8 days, during which the fish could not eat. Survival during that period was monitored by divers and the exposure length was partly decided by the fish conditions observed. After retrieving the cages, the surviving fish were collected and sacrified for liver analyses. Mortality averaged around 35% (0-50% per cage). At the time of retrieval, another set of fish (control) from each site was caught for liver exctraction on the same day. All individuals (control and experimental fish) were weighed, measured, sexed and aged; gross pathology was also noted.

RESULTS AND DISCUSSION

Preservation Study: Results indicated that the three preparation treatments exhibited significant differences (Fig. 2). Storing S10 fractions (treatment A) was more efficient in maintaining MT levels than storing homogenates (treatment B), the latter being more efficient than storing pieces of liver (treatment C). Except for treatment group A, no significant differences were found between storage periods. For treatment A, MT levels were significantly ($p<0.05$) lower after 168 days (t6) as compared to the 7 day storage (t2). Unfortunately, technical problems prevented the analysis after two days of storage (t1).

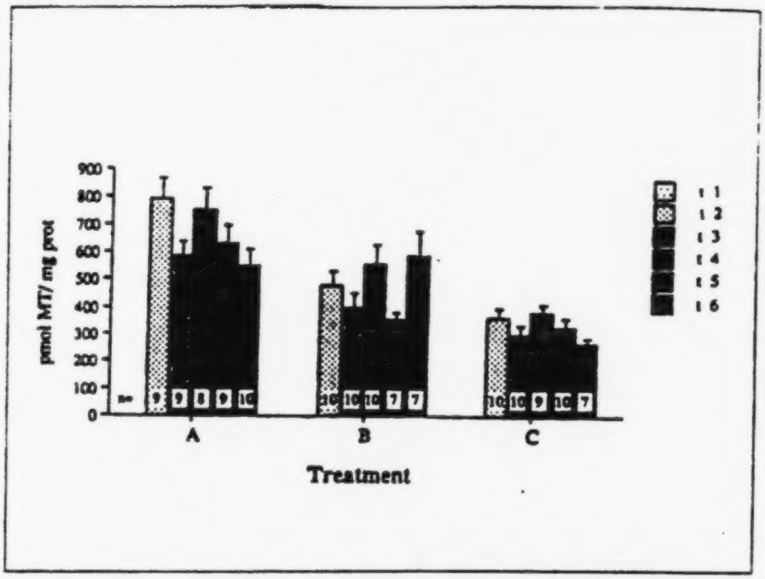

FIG. 2. Hepatic metallothionein concentrations (pmoles.mg^{-1} protein) in stored S10 (treatment A), liver homogenates (treatment B), and liver pieces (treatment C), after storage periods of 2 (t1), 7 (t2), 14 (t3), 21 (t4), 84 (t5), and 168 (t6) days. n = sample size; no results are available for t1.

of storage time is shown in Fig. 3. Concentrations are, for unknown reasons, relatively low for storage time t3 (14 days). If this low value is taken as an outlier and an artefact, then the expected value should lie more between the values of t2 and t3. This results in a trend which looks somewhat curvilinear, and a slight decrease in MT levels starts to occur after 21 days (t4), which becomes more important after 84 days (t5). MT levels decreased in the liver pieces after 84 days of storage (Fig. 2). Scheuhammer and Cherian (1991), however, reported that S10 can be stored at -85°C for several months without any significant change.

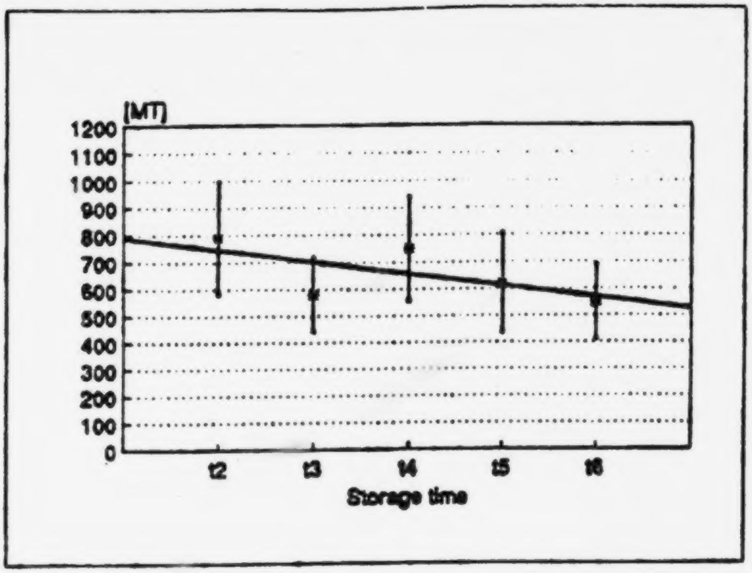

FIG. 3. Trend in mean values of hepatic metallothionein (MT) concentrations (pmoles.mg^{-1} protein), including 95% confidence limits, in stored S10 as a function of storage time.

Table 1. Mean concentrations of hepatic heavy metal (µg/g wet weight) and metallothionein[1] (nmoles/mg protein) - experiment 2; detection limit of metals and standard deviation between brackets.

	N	Cu (0.5)	Zn (0.75)	Cd (0.3)	Hg (0.2)	MT
C.u	2	63(41)	123(50)	0.10(0.08)	0.10(N=1)	0.97(0.37)[1]
C.d	10	46(25)	99(21)	0.65(0.32	0.82(0.56)	0.67(0.21)
u.u	4	56(42)	135(45)	0.81(0.82)	0.24(0.16)	0.97(0.24)
d.d	11	61(28)	111(24)	0.69(0.21)	0.71 (0.75)	0.77(0.24
d.u	7	56(18)	117(13)	0.43(0.21)	0.36(0.35)	0.78(0.26)
u.d	2	46(5)	120(0)	1.02(0.50)	0.40(0.42)	0.67(0.18)[2]

N = sample size; C = control (free-swimming) fish; u = upstream site; d = downstream site; u.u and d.d. = caged fish without transfer; d.u and u.d. = caged fish with transfer. [1]N = 8; [2]N = 17

Caged fish study: Results from the MT and heavy metal analyses are presented in Table 1. Due to high intra-station variability and, in some cases, small sample size, the observed differences between exposure treatments were rarely significant. There was, however, a certain consistency in the pattern that strongly suggested some relationship with site location. Hepatic Cd and Hg concentrations are higher at the downstream site than at the upstream site, while the opposite is observed for Cu and Zn.

MT concentrations exhibited a similar distribution pattern with respect to site as do the hepatic Cu and Zn concentrations (Fig. 4). A linear regression analysis showed the relationship to be highly significant between MT and both metals (Fig. 5). Cu and Zn were also strongly correlated between themselves (r=0.75). Both metals explained more than

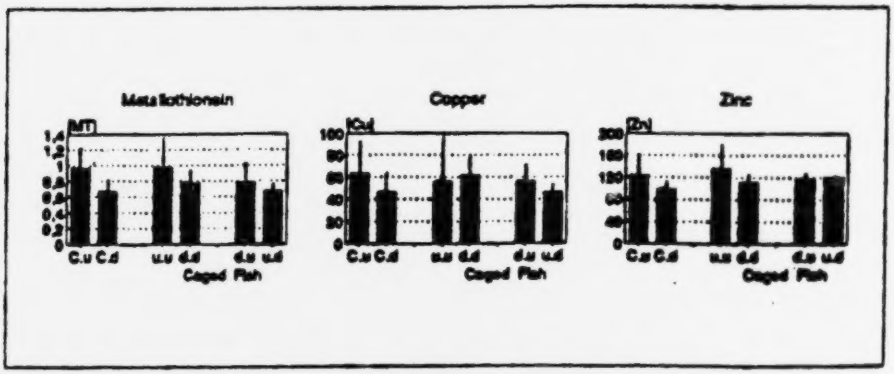

Fig. 4. Mean concentrations and 95 % confidence limits metallothionein (MT) and copper (Cu) and zinc (Zn) in liver of white sucker (nmoles.mg^{-1} protein, and µg.g^{-1} wet weight, respectively); meaning of abbreviations - see table 1.

half of the variability in MT concentrations. The data were subjected to a multiple step-wise regression analysis, where the independent variables Cu and Zn were tested individually and then together (Table 2). The analysis indicated that the best model was a multiple regression, using both independent variables, where [MT] = 0.00533[Cu] + 0.00470[Zn] (intercept is not significantly different from zero).

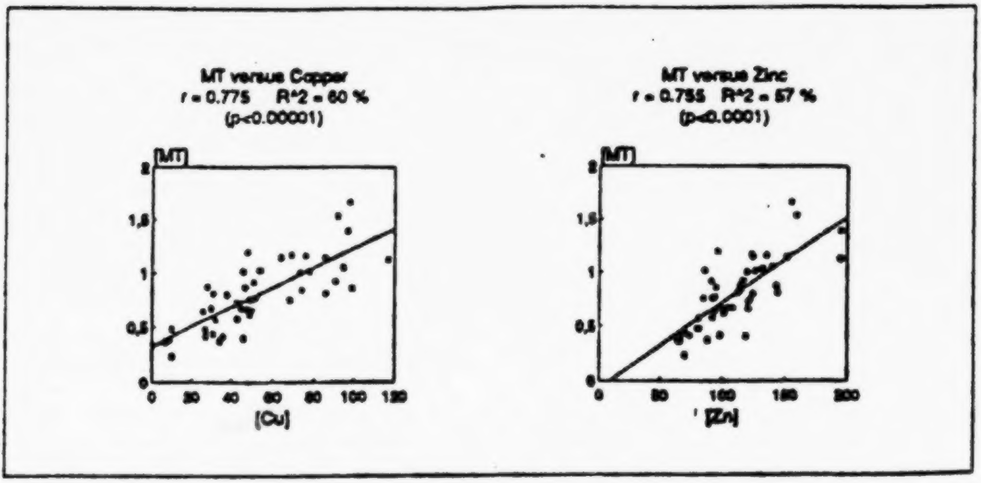

FIG. 5. Linear regression between hepatic metallothioenein (MT) concentrations (nmoles.mg^{-1} protein) and hepatic copper (Cu) and zinc (Zn) concentrations (µg.g^{-1} wet weight) in white sucker from the St. Lawrence River.

Waters concentrations of Cu and Zn (total metals, thus not all bioavailable) in the study area are about 1-4 µg/L and 1-1708 µg/L, respectively (Sylvestre 1992). Cu concentrations seemed to be slightly higher upstream than downstream. McCarter and Roch (1993) observed that at copper concentrations of 50 µg/L and higher, MT induction took place. These concentrations are higher than those cited for the St. Lawrence.

Recent data on sediments indicate that Cu and Zn concentrations are higher in the upstream site than downstream (Champoux and Sloterdijk 1988, Willsie 1993). On the other hand, mercury levels in sediments were higher downstream, which confirms our observation on hepatic Hg concentrations, while the situation for Cd is less clear. White sucker is a benthic species living at the sediment - waters interphase, feeding on benthic invertebrates. It may be that sediment heavy metal concentrations are more important than waters concentrations. It is not known, however, what role food, such as insect larvae (e.g. chironomids) and molluscs, plays in MT induction.

Table 2. Stepwise regression parameters for metallothiopnein concentrations versus copper and zinc concentrations in liver of white sucker.

step	variable in the model	coefficient	R^2
1	Copper	0,01392	0,92
2	Zinc	0,00734	0,94
3	Copper Zinc	0,00533 0,00470	0,95

These observations suggest that concentrations in the sediments or at the sediment - waters interphase, which in the latter can be expected to be much higher than in the above-lying waters column (Baudo et al. 1990), may be largely responsible for MT induction in white sucker. Klaverkamp et al. (1991) also observed that elevated levels of metal in liver of white sucker could be explained by sediment concentrations rather than direct loading from point sources. Indirectly, heavy metal levels in sediments at the upstream site of our study likely originate from upstream sources. It is not known, however, what these sources might be, either present or historically, since little industry is located upstream of the study area.

CONCLUSIONS

MT levels were maintained more efficient in the stored S10 fractions than in either the stored homogenates or liver pieces, the reason for which are unknown. Storage period seemed to cause some decrease in MT levels, although the difference was significant only for the 168 days storage period. In general, MT induction in control fish and caged fish was higher at the upstream than at the downstream site. This was contrary to the basic working hypothesis, which stated that, based on the presence of point sources in the Beauharnois area, fish populations from downstream would be exposed to higher levels of heavy metals than fish from upstream. MT concentrations varied directly and significantly with hepatic Cu and Zn concentrations, but not with Cd and Hg concentrations. Based on recent sediment data, which showed higher Cu and Zn concentrations upstream than downstream from Beauharnois, we postulated that MT synthesis in white sucker, a benthic species living and feeding at the sediment-water interphase, is induced more by sediment concentrations than by concentrations in the overlying water column.

ACKNOWLEDGEMENT

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REFERENCES

Baudo, R., J.P. Giesy and H. Muntau. 1990. Sediments: Chemistry and Toxicity of In-Place Pollutants. Lewis Publishers, Chelsea, MI.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Best. 1981. Méthodes d'analyse des métaux du cadmium, chrome, cobalt, cuivre, fer, manganese, nickel, plomb et zinc dans l'eau, les sediments, les milieux biologiques et l'air. Bureau d'études sur les substances toxiques, Ministère de l'environnement du Québec.

Champoux, L., and H.H. Sloterdijk. 1988. Etude de la qualité des sédiments du lac Saint-Louis 1984-1985. Rapport technique No. 1: Géochimie et contamination. Environnement Canada, Direction des eaux intérieures, région du Québec.

Chen, M.L., and M.L. Failla. 1989. Degradation of zinc-metallothionein in monolayer cultures of rat hepatocytes (42898). Proc. Soc. Exp. Biol. Med. 191: 130-138

Environnement Canada. 1979. Methodes d'analyses des métaux dans les sédiments, les boues et les sols. Service de la conservation et de la protection, Environnement Canada, Region du Québec.

Gagne, F. 1991. Mesure de l'induction de la metallothionéine pour indiquer la contamination des métaux lourds chez la truite arc-en-ciel (*Oncorhynchus mykiss*). Centre Saint-Laurent, Environnement Canada.

Klaverkamp, J.F., M.D. Dutton, H.S. Majewski, R.V. Hunt and L.J. Wasson. 1991. Evaluating the effectiveness of metal pollution controls in a smelter by using metallthionein and other biochemical responses in fish, p. 33-64, Chapter 2. *In*: M.C. Newman and A.W. McIntosh [ed.] Metal Ecotoxicology Concepts and Applications, Lewis Publishers, Chelsea, MI.

Klaverkamp, J.F., W.A. McDonald, D.A. Duncan, and R. Wagemann. 1984. Metallothionein and acclimations to heavy metal in fish: a review, p. 99-113, Chapter 9. *In*: V.W. Cairns, P.V. Hodson, and J.O. Nriagu [ed.] Contaminant Effects on Fisheries. A Wiley-Interscience Publication, John Wiley & Sons, Toronto, New York.

McCarter, J.A., and M. Roch. 1983. Hepatic metallothionein and resistance to copper in juvenile coho salmon. Comp. Biochem. Physiol. 74C: 133-137.

Mehra , R.K., and I. Bremner. 1985. Studies on the metabolism of rat liver copper-metallothionein. Biochem. J. 227: 903-908.

Prud'homme, J. Bureau and H.H. Sloterdijk. 1993. Effect of storage conditions of white sucker liver on some MFO activity and metallothionein concentrations. Sci. Total Environ., supplement 1993: 655-661.

Roch, M., J.A. McCarter, A.T. Matheson, M.J.R. Clark and R.W. Olafson. 1982. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39: 1596-1601.

Roesijadi, G. 1992. Review: Metallothionein in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. 22: 81-114.

Scheuhammer, A.M., and M.G. Cherian. 1986. Quantification of metallothioneins by a silver-saturation method. Toxicol. Appl. Pharmacol. 82: 417-425.

Sloterdijk, H.H., J. Bureau and A.-M. Prud'homme. 1993. Hepatic metallothionein induction in fish (White Sucker, *Catostomus commersoni*) from the St. Lawrence

River, p. 685-692. *In*: Water Pollution II, Modelling, Measuring and Prediction, L.C. Wrobel and C.A. Brebbia (Ed.), Computational Mechanics Publications, Southampton, UK.

Sylvestre, A., D. Leclerc and G. Fortin. 1992. Synthèse des connaissances sur les aspects physico-chimiques de l'eau et des sédiments du lac Saint-Louis. Rapport technique, Zones d'intérêt prioritaire n° 5 et 6. Centre Saint-Laurent, Conservation et Protection, Environnement Canada.

Willsie, A. 1993. Personl communication. Centre Saint-Laurent, Environment Canada.

METALLOTHIONEIN CONCENTRATIONS AND CYTOSOLIC METAL DISTRIBUTIONS IN INDIGENOUS FRESHWATER BENTHIC INVERTEBRATES FROM METAL-CONTAMINATED ENVIRONMENTS.

Y. Couillard[1], Y. Parisot[2] and P.G.C. Campbell[2]. [1]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, N9B 3P4; [2]INRS-Eau, Université du Québec, C.P. 7500, Ste-Foy, QC, G1V 4C7.

Metallothionein (MT) offers considerable potential as a contaminant-specific biochemical indicator of metal exposure and/or stress. Possible approaches include: (i) Direct measurement of [MT] as an indicator of prior exposure to toxic metals; and (ii) Examination of the distribution of toxic metals among cytosolic ligand pools as a means of evaluating metal stress at the biochemical level. Both approaches will be considered in this presentation. Surficial (oxic) sediments, overlying water, and specimens of the two freshwater invertebrates (*Anodonta grandis*; *Hexagenia limbata*) have been collected by divers at littoral lacustrine sites located along a geochemical gradient of pH, [Cd], [Cu] and [Zn] in an area influenced by mining and smelting activities. The animals were analyzed for Cd, Cu, Zn and metallothioneins. In addition, at selected sites subcellular metal distributions were determined by size-exclusion HPLC. Tissue concentrations of MT in both *A. grandis* and *H. limbata* were correlated with tissue Cd, but not with tissue Cu or Zn. Inter-site variations in MT concentrations were related to the free Cd^{2+} concentration at the sediment-water interface, as estimated from sediment / water sorptive equilibria - Cd^{2+} activity appears to be the key environmental factor to which organism metallothionein levels are responding in the studied lakes. It remains to be seen if the subcellular distribution of Cd also varies along the contamination gradient.

EXPERIMENTAL APPROACHES IN ASSESSING CONTAMINANT EFFECTS AT THE COMMUNITY LEVEL. SESSION CHAIR: K. LIBER

CHARACTERIZING RISKS FROM PESTICIDES IN AQUATIC ECOSYSTEMS. K.R. Solomon, Centre for Toxicology, University of Guelph, Guelph, ON, N1G 2W1.

A new paradigm for characterizing risks resulting from pesticides in aquatic ecosystems has been proposed. The process assesses levels of concern and risks to aquatic organisms based on probabilistic characterizations of susceptibility (stressor effect) in aquatic organisms and distribution of concentrations expected or found in aquatic environments (exposure characterization). This process makes better use of all the data available and allows for flexible integration with risk management options. The process is tiered to reduce unnecessary evaluation of compounds that do not pose a threat to the aquatic environment. Early stages of the tiers can be implemented on the basis of the results of laboratory tests, while higher tiers will require more acute and chronic toxicological data and computer modelling than is currently used. This approach raises questions about the usefulness of mesocosm and microcosm tests for assessing impacts at the population and community level. This presentation will focus on the use of the probabilistic approach to assessment and the generation of hypotheses which can be tested in microcosms. This will be discussed both in theoretical terms and by the use of data from field studies and observations with pesticides.

THE USE OF MESOCOSMS TO ASSESS CREOSOTE TOXICITY IN AQUATIC PLANTS: BIOMARKERS AND THEIR VALIDATION AT THE POPULATION LEVEL. [1]R.W. Gensemer, [1]K.R. Solomon and [2]B.M. Greenberg. [1]Centre for Toxicology, University of Guelph, ON, N1G 2W1; [2]Department of Biology, University of Waterloo, Waterloo, ON, N2G 3G1.

Plant bioindicators are being developed to assess the effects of polycyclic aromatic hydrocarbons (PAHs) in experimental aquatic ecosystems. Our approach is to develop and test biomarker assays that are predictive of ecological events at the population and/or community levels of organization in artificial aquatic mesocosms. PAH mixtures are being introduced into a series of aquatic mesocosms (artificial ponds) using the wood preservative creosote as a PAH source. Creosote is being applied using a regression design consisting of seven dosage levels (plus controls) ranging from 10 to 1000 ppm (total nominal additions). The growth, biomass, and species composition of phytoplankton, periphyton, and stocked macrophytes (*Lemna gibba, Myriophyllum* sp.) will then be measured throughout the growing season, and compared to one or more biomarker assays used to detect PAH contamination. Preliminary lab results using chlorophyll fluorescence induction (an index of photosystem II quantum yield) in *L. gibba* suggest that PAHs can significantly inhibit photosynthesis 1-4 hours after exposure, and at concentrations below those required to affect population growth. We will be verifying the performance of this photosynthesis biomarker using plant populations in our enclosures, and comparing these results with creosote effects at both the population and community levels.

SEDIMENT CONTAINERS: A USEFUL TOOL FOR THE STUDY OF CONTAMINANT EFFECTS IN NATURE. L. Hare. INRS-Eau, Université du Québec, Sainte-Foy, QC, G1V 4C7.

The biological impact of sedimentary contaminants is difficult to evaluate in the laboratory, partly because the bioavailability of contaminants tends to be altered when the sediments are removed from nature. Experiments using sediment-filled containers could be a useful means of validating the results of laboratory bioassays and of studying contaminant biogeochemistry *in situ*. An example is presented in which experimentally contaminated sediments were used *in situ* to evaluate the utility of the AVS (acid volatile sulfide) model in predicting the bioavailability and toxicity of Cd. The AVS model has been developed in the laboratory and had not been previously tested in nature. Lacustrine sediment was contaminated with various concentrations of Cd, and placed in containers on the lake bottom. Cadmium and AVS concentrations were measured in the sediments and in insects colonizing them. The immigration rates of the animals into the containers were also measured. The experimental results obtained suggest that the *in situ* approach tested was effective in evaluating the efficacy of the AVS model in predicting the biological effects of metal-contaminated sediments in nature.

USING STABLE ISOTOPES TO DESCRIBE ORGANOCHLORINE BIOACCUMULATION IN TWO DISTINCTIVELY DIFFERENT GREAT LAKES FOOD WEBS. R.M. Kiriluk[1], M.R. Servos[1], D.M. Whittle[1], G. Cabana[2] and J.B. Rasmussen[2]. [1]Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, ON, L7R 4A6; [2]McGill University, Department of Biology, Montreal, QC, H3A 1B1.

A high degree of variability has been observed in organochlorine concentrations in biota from systems within the Great Lakes basin with comparable water and sediment contaminant concentrations. It has been proposed that these between-lake differences may be attributed to the food chain length as well as the degree of predator-prey interactions within these systems. These relationships have been traditionally defined by labour intensive analysis of gut contents. This gives a measure of what is consumed at one point in time and does not provide a time integrative measure of food chain dynamics. Therefore, the difficulty in accurately describing food webs in nature has resulted in the development of contaminant bioaccumulation models that do not account for the impact of food chain dynamics. Biota typical of two distinctively different food webs, Lake Ontario and Lake Erie, were analyzed for stable isotopes of nitrogen and carbon and select organochlorines. The stable isotopic descriptions of these two food webs illustrates the complexity of the predator/prey interrelationships associated with these systems. Furthermore, there was a significant correlation between the bioaccumulation of select organochlorines and the trophic level of an organism as defined by ^{15}N through both food webs.

POSTERS PRESENTATIONS/SÉANCES AFFICHES

UTILIZING PCB CONGENERS AS TOOL TO DETERMINE TRANSPORT PATHWAYS WITHIN A LARGE RIVER SYSTEM.* J. Biberhofer[1], M.E. Comba[2], S.M. Backus[2] and K.L.E. Kaiser[2]. [1]Ecosystem Health Division, Environment Canada, Burlington, ON, L7R 4A6; [2]National Water Research Institute, Environment Canada, Burlington, ON, L7R 4A6.

Located at the eastern end of the international section of the St. Lawrence River, are two industrial centres which discharge contaminants into the St. Lawrence River. Multimedia samples were collected to support the design of a long term monitoring program to measure transboundary contaminant movement. PCBs, a primary contaminant within the region, were used as indicator compounds utilizing congeners measured by high resolution gas chromatography. Large volume water samples, suspended particulate samples, bottom sediments and freshwater mussels (*Elliptio complanata*) were collected and analyzed from a series of sites distributed within the impacted region as well as from an upstream control site. Congener profiles from sites downstream of the discharges differed from those of the control site in both increased number of congeners detected and the relative concentrations. Introduced congeners were found throughout the range of isomer groups but were more pronounced in the lower chlorinated range.

IN SITU* BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS BY ZEBRA MUSSELS. H.A. Morrison, R. Lazar and G.D. Haffner. Great Lakes Institute for Environmental Sciences and Department of Biology, University of Windsor, Windsor, ON, N9B 3P4.

Uptake rate constants (k_u), representing chemical accumulation by *Dreissena polymorpha* Pallas from food and water, were measured *in situ* for PCBs ranging in octanol/water partition coefficients ($\log K_{ow}$) from 5.60 to 7.36. Accumulation curves indicated that PCB uptake was rapid and that lower K_{ow} congeners achieve steady-state faster than higher K_{ow} congeners. Uptake rate constants were not significantly related to $\log K_{ow}$. Lipid-normalized bioaccumulation factors ($\log BAF_{lip}$) were less than predicted bioconcentration factors (logBCF) indicating either trophic dilution or an overestimate of the freely-dissolved aqueous phase occurred for congeners with a $\log K_{ow} > 6.3$. Additionally, measured water concentrations, determined by the hexane extraction method, were compared to calculated water concentrations, based on zebra mussel body burdens. The hexane extraction method of unfiltered water provided a good estimate of the concentration of chemical available to zebra mussels. This study also suggests that contaminated food is a major source of chemical to mussels.

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*These papers were presented at a special session hosted by the Laurentian Chapter, Society of Environmental Chemistry and Toxicology.

ACCUMULATION OF INDUCER(S) OF FISH MIXED FUNCTION OXYGENASE (MFO) FROM PULP MILL EFFLUENTS AND BLACK LIQUOR BY SEMI-PERMEABLE MEMBRANE DEVICES (SPMDS).* J.L. Parrott[1], D.E. Tillitt[2], P.V. Hodson[1], J.Y. Wilson[1], D.T. Bennie[1], J.N. Huckins[2] and J.D. Petty[2]. [1]National Water Research Institute, Environment Canada, 867 Lakeshore Rd, Burlington, ON, L7R 4A6; [2]United States Department of Interior, National Fisheries Contaminant Research Center, 4200 New Haven Rd., Columbia, MO, 65201.

SPMDs are triolein-containing, thin polyethylene membranes that can be used to concentrate hydrophobic compounds from air, water, sediment and soil. We wanted to see if they would accumulate the MFO-inducing chemicals from two pulp mill effluents and black liquor from a third mill, and to compare their accumulation to rainbow trout (*Oncorhynchus mykiss*) exposed similarly. Dialysates of SPMDs, exposed for 6 d, either in the lab in barrels of effluent or in the field in an effluent stream, induced ethoxyresorufin-O-deethylase (EROD) activity in a fish (*Poeciliopsis lucida*) hepatoma cell line, PLHC-1, to 580-2594 pg TCDD equivalents/g triolein, while control water SPMD extracts showed no EROD induction. Dialysates of SPMDs exposed for 4 d to 0.032% black liquor from a third pulp mill or β-naphthoflavone (BNF; 10 μg/L) induced PLHC-1, and rainbow trout exposed to similar solutions showed 56 (black liquor) or 12 (BNF) fold increase in EROD activity. Both fish and SPMDs were able to remove a small portion of the inducers from black liquor or BNF solutions in 1 d, with induction decreasing to 24 or 41 fold for the fish- or SPMD-stripped solutions, respectively.

INDUCTION OF EROD AND EMBRYOTOXICITY IN FISH EXPOSED TO PHYTOCHEMICALS.* Y. Kiparissis[1] and C.D. Metcalfe[1]. [1]Environmental and Resource Studies Program, Trent University, Peterborough, ON, K9J 7B8.

It is possible that secondary natural constituents of trees induce the mixed function oxidases and cause embryotoxic effects in fish. This study evaluated the ability of 4 phytochemicals to induce hepatic ethoxyresorufin-O-deethylase (EROD) in rainbow trout and (*Oncorhynchus mykiss*) and to be toxic to embryos of Japanese medaka (*Oryzias latipaes*). Immature rainbow trout were intraperitoneally injected with 7-methoxycoumarin, 2-hydroxy-2,4,6-cycloheptatrienone (tropolone), b-carboline (harmane), and flavone at a dose of 10 mg/g. Tropolone and flavone were moderate inducers of hepatic EROD, whereas harmane was a weak EROD inducer. All 4 phytochemicals were highly toxic to medaka embryos at concentrations between 1 to 10 mg/ml.

DIOXIN TOXIC EQUIVALENT CONCENTRATIONS IN LAKE TROUT (*Salvelinus namaycush*) FROM LAKE ONTARIO AND LAKE SUPERIOR AS DERIVED WITH LIVER CELL LINES FROM RAINBOW TROUT AND RAT.* J.J. Whyte, M.R. Van Den Heuvel, J.H. Clemons, N.C. Bols and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

Chemical residues extracted from the livers of Lake Trout (*Salvelinus namaycush*) sampled from two Lake Superior sites (Jackfish Bay and Black Bay) and one Lake Ontario site (Glenora, Ontario) were exposed to the RTL-W1 rainbow trout liver cell line and the H4IIE rat hepatoma cell line. These two bioassays were used to measure the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent concentrations (TECs) of the liver residues as indicated by their relative abilities to induce 7-ethoxyresorufin-o-deethylase (EROD). This estimates the burden or accumulation by fish of compounds that could act through the Ah receptor and are potentially toxic. Response differences between the two cell lines as well site to site variation within cell lines were examined. Extracts from Lake Ontario exposed to RTL-W1 cell were found to have a higher mean TECs than those from Jackfish Bay. The reference site, Black Bay, was found to have the lowest TEC values. The RTL-W1 cell line bioassay was found to have excellent utility for rapid determination of TECs and should be a generally suitable tool for estimating the toxic potency of environmental samples.

THE EFFECT OF PCB CONGENERS ON THE *IN VITRO* EROD INDUCTION OF TCDD IN A FISH AND MAMMALIAN CELL LINE.* J.H. Clemons, D.G. Dixon and N.C. Bols. Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

In previous studies, we have compared the relative potencies of selected congeners of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls between a rainbow trout liver, RTL-W1 and a rat hepatoma, H4IIE, cell line, by a system of toxic equivalent factors (TEFs). These factors were derived based on an *in vitro* bioassay approach where induction of ethoxyresorufin-o-deethylase (EROD) was the sensitive biochemical response. To use these factors for prediction of environmental mixture toxicity, an assumption of additivity and chemical non-interaction is applied. The hypothesis of this study, through the use of the isobolographic method of analysis, was that potent PCBs, theorized to induce the EROD response through the same mechanism, will act non-interactively when mixed in binary combinations with TCDD. For a 24 hours, the cells were subjected to a fixed dose of either combinations of TCDD, in a concentration less than it's EC50, in combination with 7 incremental doses of TCDD or PCB. The EROD response to the individual PCBs differs greatly between cell lines. RTL-W1 does not induce when subjected to PCBs 105, 156 and 169 but does to PCBs 77, 81, 118, and 126. In contrast, H4IIE responds to all listed congeners. Synergistic, antagonistic or additive responses between PCBs and TCDD will be demonstrated between the two cell lines.

USE OF RAINBOW TROUT LIVER ESTROGEN RECEPTORS TO EVALUATE THE ESTROGENIC ACTIVITY OF XENOBIOTIC COMPOUNDS.* L. Tremblay and G.J. Van Der Kraak. Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1.

An assay to screen for compounds that could be estrogenic to fish was developed. Most of these compounds work as mimics of the key steroid hormone 17ß-estradiol (E_2) by interacting with the receptor. We isolated E_2 receptors from immature E_2 primed rainbow trout (*Oncorhynchus mykiss*) liver, a target organ for E_2. E_2 binding activity was estimated by using a radioassay using $[^3H]E_2$. The receptors identified were highly specific, saturable and exhibited high affinity and low capacity. A competition assay was developed where a constant amount of $[^3H]E_2$ was incubated with increasing concentrations of a competitor. Known estrogenic compounds including diethylstillbestrol (DES) and genistein (a phytoestrogen) were shown to compete with E_2 for the receptors. We tested detergent samples (nonylphenols) found in treated industrial and municipal sewage waters as well as in pulp and paper mill effluents. Using our system, we found that some nonylphenols have estrogenic activity at concentrations relevant to what has been found in nature.

IMPACT OF A THREE DAY RECOVERY PERIOD ON NON-SPECIFIC STRESS INDICATORS AND SEX STEROIDS AFTER ROUTINE CAPTURE OF WHITE SUCKER EXPOSED TO BLEACHED KRAFT PULP MILL EFFLUENT.* J.J. Jardine[1], G.J. Van Der Kraak[1] and K.R. Munkittrick[2]. [1]Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1; [2]GLLFAS, Department of Fisheries and Oceans, Burlington, ON, L7R 4A6.

The present study was conducted to evaluate whether a recovery period of 1 or 3 days after capture would be applicable when using markers of physiological fitness (plasma cortisol, glucose, lactate, total protein and the reproductive steroids testosterone, 11-ketotestosterone, and 17ß-estradiol) to assess the impacts of BKME on white sucker. There was limited evidence for recovery in prespawning fish, however it was difficult to differentiate whether the biochemical changes seen (decreases in plasma cortisol, glucose, and lactate and increases in plasma 17ß-estradiol and testosterone at the BKME or reference sites) were a result of recovery or reflected the normal physiological changes expected in white sucker during preovulatory period. Fish captured in the fall showed no recovery from capture and handling stress; plasma levels of cortisol, glucose, and lactate were elevated and remained high while the reproductive steroids decreased during the recovery period. Therefore, holding white sucker for 1 or 3 days to recover from the stress of capture in field studies was not applicable when these fish were captured by gill net in the fall. This study reinforced the necessity to limit the amount of acute physical stress fish are exposed to prior to sampling, particularly when measuring markers of physiological fitness as indicators of exposure or responses of fish chronically exposed to environmental contaminants.

BIOAVAILABILITY OF THE PYRETHROID INSECTICIDE, TEFLUTHRIN, TO BENTHIC INVERTEBRATES: *Tubifex tubifex* AND *Chironomus riparius*.* H.D. Roshon[1], K.E. Day[2], J.C. Hall[1] and N.K. Kaushik[1]. [1]Department of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1; [2]National Water Research Institute, Environment Canada, Burlington, ON, L7R 4A6.

The interaction of hydrophobic chemicals with sediments and their effect upon benthic organisms has recently become an important area of study for aquatic toxicologists.

Lipophilic chemicals are strongly adsorbed to soil particles, allowing sediments to become a chemical sink. These chemicals are then available for uptake by benthic organisms through ingestion and/or absorption through the cuticle. *Tubifex tubifex*, an oligochaete worm, and *Chironomus riparius*, a non-biting midge, are two of the benthic species currently under investigation. Tefluthrin is a highly lipophilic synthetic pyrethroid insecticide used to control corn rootworm. This project has been designed to address the toxicity of tefluthrin to these two species under various substrate and temperature conditions. Acute 48-hour tests, in Guelph well water, using both the technical chemical and the emulsifiable concentrate formulation, show that tefluthrin is highly toxic to both the adult oligochaete worms and the larval midges. Acute tests with tubificids at 18°C, 23°C, and 28°C, show that tefluthrin has a slight negative temperature coefficient. Chronic 28-day tests with adult tubificids in Longpoint sediment demonstrate that this insecticide seriously impairs reproduction at both 18°C and 23°C. Tefluthrin also decreases the growth and survival of chironomids during 10-day tests in sediment, silica sand, or a sediment/silica sand mix. There is little difference between the toxicity of tefluthrin in Longpoint sediment (10% organic carbon) and in a Longpoint sediment/silica sand mix (5% organic carbon). The toxicity of tefluthrin in silica sand (organic carbon = <1%), however, is equal to the toxicity of tefluthrin in the acute tests.

DEVELOPMENT OF A SEDIMENT BIOASSAY FOR DETERMINING THE BIOAVAILABILITY AND EFFECTS OF PAHS ON FISH.* K.R. Munkittrick[1], B. Blunt[1], M. Leggett[1], S. Huestis[1] and L.H. McCarthy[2]. [1]GLLFAS, Department of Fisheries and Oceans, Burlington, ON, L7R 4A6; [2]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.

For the past six years, we have been examining the relationship between induction of fish liver detoxification enzymes and reproductive abnormalities which occur in fish exposed to effluents from pulp and paper mills. Additional recent work has shown that similar biochemical dysfunction occurs after exposure to other compounds, including some pesticides, PCBs and PAHs. To further examine this, we have initiated the development of a sediment bioassay capable of demonstrating the biochemical problems in fish. These experiments demonstrated that biochemical responses were evident in laboratory fish within 4 d of exposure to contaminated sediments. Further testing demonstrated that fish were responding to chemicals from the sediments and not from overlying water, and a survey of sediment from 13 contaminated areas demonstrated that fish responses paralleled PAH levels in the sediments, as opposed to PCBs. The protocol has been further refined to describe quantity of sediment required, duration of testing and species preferences. This test will enable us to closely study the biochemical effects of exposure to contaminated sediments, and the biochemical responses measured in this bioassay parallel responses seen in wild fish collected at some of the sites. The protocol could also be used to prioritize areas of contamination and to evaluate dredging impacts and remediation success.

USING BIOLOGICAL INFORMATION FOR SEDIMENT MANAGEMENT DECISIONS; CASE STUDY: COLLINGWOOD HARBOUR.* G. Krantzberg. Ontario Ministry of Environment and Energy, 40 St. Clair Ave. W., Toronto, ON, M4V 1M2.

The significance of metal residues in biota and the effects of contaminants in sediment on organism health are not easily predicted. Comparisons of biotic responses and tissue residues in exposed organisms with those of organisms from locations remote from pollutant sources is a means of identifying adverse biological effects. In the case of Collingwood Harbour, tissue residues of contaminants in field laboratory organisms were comparable to concentrations observed in organisms colonizing reference sediment, although sediment concentrations exceeded provincial guidelines. Sediment bioassays using sensitive endpoints indicate no observable impacts of harbour sediment on the biota, however, *Tubifex tubifex* reproduction was impaired in sediment from the Canada Steamship Lines (CSL) property. With the exception of the CSL sediment, the bioassay information, coupled with field observations on native benthic invertebrates, young of the year spottail shiners, and introduced mussels provides multiple lines of evidence that support the conclusion that concentrations of biologically available contaminants in Collingwood Harbour sediment are not of toxicological significance. In the CSL property and proximity, a program to remove sediment that elicited adverse biological responses coupled sediment removal with the opportunity to demonstrate new innovative technology that could have potential for sediment rehabilitation in more seriously contaminated areas throughout the Great Lakes.

PERSPECTIVE SPATIO-TEMPORELLE DU FACTEUR D'ENRICHISSEMENT (FEA) DES MÉTAUX TRACES DANS LES SÉDIMENTS DU SAINT-LAURENT.* S. Lorrain[1], R. Carignan[2] et K. Lum[1]. [1]Centre Saint-Laurent, Environnement Canada, Montréal, QC, H2Y 2E7; [2]INRS Eau, Sainte-Foy, QC, G1V 4C7.

Les sédiments ont été échantillonnés en 1990 et 1992 à 22 sites localisés entre Cornwall et Trois-Rivières; un tronçon de 100 km de longueur sur le fleuve Saint-Laurent. Les profils de métaux traces ont été étudiés afin de décrire l'historique de la pollution pour ce tronçon du fleuve. On observe des différences importantes des tendances des profils entre les stations, suggérant l'effet de sources distinctes. À cause des conditions d'écoulement du fleuve qui favorisent le transport préférentiel des contaminants dans des masses d'eau distinctes, il est impossible d'obtenir un profil cumulatif de la contamination provenant de plusieurs sources. À quelques sites, des sédiments d'âge supérieur à 130 ans (pré-industriels) ont été analysés pour déterminer les teneurs naturelles en métaux traces. Les concentrations obtenues ont servi à établir une série d'équations où la teneur naturelle d'un élément trace est exprimée en fonction du contenu en argile. Ces équations ont ensuite servi à calculer un facteur d'enrichissement anthropique (FEA) des métaux traces qui tient compte de l'effet des changements granulométriques sur les concentrations. En général, les profils du FEA montre une tendance relativement constante des apports anthropiques au lac Saint-François depuis les 20 ou 30 dernières années, alors qu'au nord du lac Saint-Louis une nette tendance à la baisse est observée depuis les 3 dernières décennies. Ce dernier site est probablement représentatif des apports provenant des Grands Lacs lorsque l'on considère la localisation de la station et le patron d'écoulement. Vers l'aval, les profils du FEA montre une augmentation pour la plupart des contaminants provoquée par les apports de sources locales et/ou un effet d'addivité des sources. En considérant la localisation du site du delta de Sorel et le patron de circulation, le signal observé de la pollution est probablement représentatif des apports provenant de l'île de Montréal. À ce site, les profils du FEA pour les différents métaux traces indique une augmentation des apports provenant de l'île de Montréal, à l'exception du Pb dont la diminution s'est amorcée vers la fin des années 1960. Pour l'ensemble des sites échantillonnés, la valeur du FEA pour le Cr et le Ni approche de l'unité, soulignant l'absence de problème sérieux de contamination par ces deux éléments.

THE USE OF RISK ASSESSMENT TECHNIQUES TO DEVELOP ACCEPTABLE CONCENTRATIONS OF BIOACCUMULATIVE CHLORINATED ORGANIC CHEMICALS IN AQUATIC BASED ECOSYSTEMS. I: FOR THE PROTECTION OF AQUATIC SPECIES.* M.E. Starodub[1], P.A. Miller[1], J.P. Giesy[2] and R.F. Willes[1]. [1]CanTox Inc., 2233 Argentia Road, Suite 308, Mississauga, ON, L5N 2X7; [2]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, 48824-1222.

Environmental quality criteria are benchmarks that indicate whether or not environmental concentrations present measurable risks to ecosystem health. Regulatory methods for the derivation of water quality criteria have been based on acute and chronic toxicity data generated primarily from waterborne exposure assays in phytoplankton, invertebrates and fish. While more recent efforts aimed at the development of sediment quality criteria have focused on acute and chronic toxicity of contaminated sediments to benthic invertebrates. Neither methodology adequately addresses the potential for food chain transfer of persistent bioaccumulative organic chemical through relevant exposure pathways. A thorough review of the aquatic toxicology literature for chlorinated organic chemicals found a notable lack of information on no-observed effect levels (NOELs) for dietary and sediment exposure routes and for dietary chemical assimilation efficiencies in aquatic species. Future efforts should be directed at filling these important data gaps and understanding the interactions of aquatic food chains with sediments. Food chain modelling techniques that integrate information from such studies can aid in the development of environmental quality criteria for persistent bioaccumulative chemicals derived for the protection of aquatic ecosystems.

THE USE OF RISK ASSESSMENT TECHNIQUES TO DEVELOP ACCEPTABLE CONCENTRATIONS OF BIOACCUMULATIVE CHLORINATED CHEMICALS IN AQUATIC BASED ECOSYSTEMS. II: FOR THE PROTECTION OF PISCIVOROUS WILDLIFE.* P.A. Miller[1], M.E. Starodub[1], J.P. Giesy[2] and R.F. Willes[1]. [1]CanTox Inc., 2233 Argentia Road, Suite 308, Mississauga, ON, L5N 2X7; [2]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, 48824-1222.

During the past two decades societal concerns over potential adverse effects of bioaccumulative chlorinated chemicals on human health and the environment have been increasing. Bioaccumulative chlorinated organic chemicals have been implicated in a variety of adverse effects observed in piscivorous birds on the Great Lakes. Using a food chain approach, risk assessment methods including modelling techniques can be used to derive acceptable concentrations of bioaccumulative chemicals for the protection of the upper trophic levels. Wildlife receptors are selected based on exposure characteristics and/or population concerns (*e.g.*, endangered species) and predator-prey relations are defined. Trends in concentrations of bioaccumulative chemicals in wildlife will be discussed in terms of exceeding health-based limits.

A QUANTITATIVE HUMAN HEALTH RISK ASSESSMENT OF POSSIBLE RISKS ASSOCIATED WITH THE CONSUMPTION OF FISH FROM A LAKE ADJACENT TO A PROPOSED MUNICIPAL SOLID WASTE INCINERATOR.* C.E. Moore[1], E.A. Sigal[2], P.A. Miller[2], L.A. Haighton[2] and R.F. Willes[2]. [1]CanTox Inc., 5871 Spring Garden Road, Halifax, NS, B3H 1Y2; [2]CanTox Inc., 2233 Argentia Road, Suite 308, Mississauga, ON, L5N 2X7.

A quantitative human health risk assessment on the possible health risks associated with consumption of fish from a lake located near a proposed Waste-To-Energy Facility was conducted as part of the provincial Environmental Impact Assessment process. This assessment involved estimating possible chemical input into the lake system through direct atmospheric deposition onto the lake and through indirect runoff from the lake watershed. The environmental fate and behaviour of selected organic chemicals (PCDD/PCDF, PCB and chlorinated benzenes and phenols) within the lake was estimated using the EXAMS II computer model. Accumulation within a representative aquatic food chain was modelled using a dynamic food chain model. Possible human health risks associated with consumption of fish was calculated using the Exposure Risk Assessment Model (ERAM), and was calculated in conjunction with possible exposures from other sources, such as inhalation of air, ingestion of local garden produce, ingestion of soils and drinking water. A similar assessment was conducted for mercury emissions, using a simplified approach involving assumptions related to conversion rates to methyl mercury and bioaccumulation factors reported in the scientific literature. Methodology and results will be presented and discussed. Results of the study indicated that consumption of local fish which had been exposed to selected chemicals released from the proposed facility would not be associated with measurable human health effects.

LES OBJECTIFS ENVIRONNEMENTAUX DE REJET POUR LES SOURCES PONCTUELLES ET LE CADRE LÉGAL AU QUÉBEC.* I. Guay. Service d'évaluation des rejets toxiques, Direction des écosystèmes aquatiques, ministère de l'Environnement et de la Faune, 930, chemin Sainte-Foy, 2e étage, Québec, QC, G1S 2L4.

Le ministère de l'Environnement et de la Faune (MEF) a entre autres deux grands objectifs: préserver la diversité, la productivité et la pérennité des écosystèmes, et diminuer l'exposition des personnes et des espèces aux substances toxiques. Afin d'atteindre ces objectifs, le MEF a développé une approche de protection du milieu aquatique basée sur la protection des usages de l'eau. Cette approche, qui se veut préventive d'un point du vue environnemental, a l'avantage d'être quantitative et de guider les choix d'interventions d'assainissement pour le contrôle des rejets liquides toxiques. Dans ce contexte, l'affiche a pour but de présenter les grandes étapes de la procédure de définition des objectifs environnementaux de rejet. Voulant protéger ou récupérer les usages de l'eau, la procédure s'appuie sur la protection du milieu aquatique contre les effets toxiques des substances chimiques en exigeant le respect des critères de qualité de l'eau à la limite d'une zone de mélange dans le cours d'eau. Par la suite, le cadre légal de l'application de cette approche sera présenté en insistant sur son utilisation dans le cas de nouveaux établissements industriels et dans le nouveau cadre d'application qu'est l'attestation d'assainissement. Enfin, on procédera à une brève comparaison entre l'approche du MEF et celle proposée par le Great Lakes Water Quality Initiative (G.L.W.Q.I.) pour unifier les méthodes en vigueur parmi les états américains du pourtour des Grands Lacs.

A PRESERVABLE STAINING PROCEDURE TO DETECT TOXICANT IMPACTS ON ALGAE *(Selenastrum capricornutum)* USING FLOW CYTOMETRY. M.J. Faber[1], L.M.J. Smith[2] and H.J. Boermans[2]. [1]Department of Environmental Biology, [2]Department of Biomedical Sciences, University of Guelph, Guelph, ON, N1G 2W1.

Over the last several years, the use of flow cytometry to assess the impacts of toxicants on algae has increased. A cell viability determination technique (using fluorescein diacetate) and a preservation technique specifically designed for flow cytometric analysis have been developed. This paper expands on these developments. A simple technique has been developed to assess the number of live and dead cells within a culture. An added feature to this technique is the ability to cross compare chlorophyll *a* fluorescence (as a viability indicator) with live/dead cell counts. After staining, cells can be fixed in a glutaraldehyde and frozen in liquid nitrogen so that assessment at a later date is possible. The staining procedure involves 2 molecular dyes. Fluorescein diacetate is used as an indicator of live cells and ethidium homodimer-1 is used as an indicator of dead cells. Since these dyes fluoresce at 525 nm and 620 nm repectively the maximum autofluorescence of Chlorophyll *a* at 675 nm is maintained and can be used to assess cell viability. Results indicate that in a population of *Selenastrum capricornutum* containing approximately 50% live cells and 50% dead cells, 45.6% stained as live cells and 47.6% stained as dead cells. Using chlorophyll *a* fluorescence, 49.8% fluoresced at a high intensity indicating live cells and 50.2% fluoresced at a low intensity indicating dead cells. Expansion of this method for use in field situations is currently under investigation.

LA SÉLECTIVITÉ MÉTALLIQUE DÉMONTRÉE PAR QUELQUES BRYOPHYTES INDIGÈNES. C. Schiltz, G. Samson et L. Pazdernik. Université du Québec à Trois-Rivières, Département de chimie-biologie, C.P. 500, Trois-Rivières, QC, G9A 5H7.

Un intérêt récent a été porté aux bryophytes comme bioindicateurs considérant leur

grande capacité d'accumuler les polluants plus particulièrement les substances métalliques. Cependant, peu de travaux ont été jusqu'à maintenant réalisés au Québec. Le but de ce projet est d'effectuer le bilan métallique chez des bryophytes provenant de diverses régions de la province de Québec. Même si la majorité des éléments chimiques sont présents dans l'environnement, certains le sont en plus grande quantité. De plus, il semble que la capacité de bioaccumulation soit différente selon l'espèce, la portion de la plante étudiée et le métal analysé. Pour réaliser cet objectif, le calcium, magnésium, cuivre, cadmium, manganèse, fer, nickel cobalt et chrome furent analysés. Considérant que les bryophytes possèdent un système vasculaire très simple, que leurs feuilles n'ont qu'une cellule d'épaisseur et qu'elles n'ont pas de racines, le processus d'accumulation est surtout centré sur des échanges ioniques(adsorption); les cellules ayant une charge négative alors que les métaux sont chargés positivement. Nos résultats démontrent que les mousses provenant de la rivière Caché(Québec) sont fortement contaminées au cobalt. Quant au cuivre, cet élément est présent en concentrations élevées à St-Alexis des Monts, Loretteville et Québec. *Fontinalis dalecarlica* Schimp. ex B.S.G. et*Platyhypnidium riparioïdes* (Hedw) Dix. semblent l'accumuler plus facilement que les autres espèces; c'est qu'il y a sélectivité selon les espèces. A Loretteville par exemple, *P. riparioides* est 300 fois plus contaminée en cuivre que *Brachytécium rivulare* provenant du même site. A l'exception du calcium et du magnésium, nos résultats concordent avec ceux cités dans la littérature si on compare la concentration dans les tiges et dans les feuilles.

DIFFERENTIAL ACCUMULATION AND COMPLEXATION OF CADMIUM BY METAL TOLERANT AND SENSITIVE WHEAT CULTIVARS (*Triticum aestivum*). D.Y. Chan and B.A. Hale. Department of Horticultural Science, University of Guelph, Guelph, ON, N1G 2W1.

The role of plants in the environmental fate of heavy metals is important to address, especially with regard to their availability to humans and animals. Heavy metals contained in the harvested portion of grains increase the potential for human exposure, while residues in the remaining portions can enter the food web via animal feed and tilling processes. Cadmium (Cd) has received a great deal of attention due to its acute and chronic human toxicities and its tendency to bioaccumulate in organs and tissue. Most of the cadmium present in human tissue is of dietary origin. Two cultivars of wheat (Kyle and Sceptre) demonstrating differential accumulation are being used to assess the uptake and translocation of cadmium. The fate of cadmium at four separate life stages is being investigated hydroponically. The addition of a spiked solution containing a stable isotope of Cd allows for the determination of routes of uptake and/or translocation and the form in which Cd is complexed. The concentration of Cd will be 0.09 μM in the nutrient solution. This concentration has been used by others to approximate Cd levels in contaminated soils. An inductively coupled plasma atomic emission spectrometer and high performance liquid chromatography will be used to quantify the amount of Cd present in the roots, stems, leaves and grain and to identify the complexed forms of Cd present, respectively. Data will be used to compare and evaluate current translocation models, leading to the determination of inclusion mechanisms which may help explain differential accumulation.

A STEADY STATE SYSTEM FOR FOLIAR EXPOSURE OF PLANTS TO POLYCYCLIC AROMATIC COMPOUNDS (NAPHTHALENE). S. Love and B.A. Hale. Department of Horticultural Science, University of Guelph, Guelph, ON, N1G 2W1.

A steady state exposure system was developed to study the effect of gaseous polycyclic aromatic compounds (PAC) on horticultural species. The system also provides a means of evaluating numerous theoretical models which attempt to characterize the flux of organic pollutants to vegetation (e.g. fugacity), or plant response based on chemical characteristics of the compound (e.g. QSAR). Naphthalene, which has the simplest chemical structure of all PACs, was chosen as the model PAC and exposed to 21 day old *Beta vulgaris* cv. White King (Swiss chard). A split stream flow through system was developed, which allowed the insertion of a chilled naphthalene permeation device, in series with the inflow air line. Naphthalene and air flow rates were electronically controlled and remixed prior to entering a 7L plexiglass exposure cuvette. Gaseous naphthalene concentrations consisted of: 0, 10, 100, and 1000 nl/L. Exposures were conducted either during the day or night, for an eight hour period over four days. No significant growth response of Swiss chard was noted. Evaluation of physiological responses are continuing.

ÉVOLUTION TEMPORELLE DES MÉTAUX BIOLOGIQUEMENT ESSENTIELS DE LA FLORE MUSCINALE LORS D'ÉTUDE EN LABORATOIRE. G. Samson, G. Vaillancourt et L. Pazdernik. Université du Québec à Trois-Rivières, Département de chimie-biologie, C.P. 500, Trois-Rivières, QC, G9A 5H7.

Jusqu'à présent, diverses expérimentations ont été effectuées pour comprendre les interactions entre les ions présents dans un organisme vivant. Dans cette portion d'étude, l'objectif est de suivre la synergie de certains cations chez ces végétaux. Le dosage des métaux essentiels(calcium, magnésium, potassium, sodium et zinc) est effectué afin d'évaluer leur comportement face à l'introduction du cadmium dans le milieu. Ces mesures permettront de vérifier, s'il y a lieu, l'effet compétitif entre ces éléments biologiquement essentiels et le contaminant. Pour mieux suivre l'évolution des cations à l'intérieur des bryophytes, nous avons utilisé les genres Fontinalis et Platyhypnidium qui sont des mousses dulcaquicoles ayant une morphologie différente. Il est intéressant de constater que *P. riparioïdes* est détectable par la couleur rouge de sa flamme en spectro-absorption atomique, caractéristique d'une teneur accrue en calcium. Nos résultats ont démontré que les mousses aquatiques étudiées répondent à une décontamination par une augmentation de la concentration en calcium. En effet, le calcium est passé de 2 à 5 µg/g pour Fontinalis et de 2,5 à 10 µg/g dans le cas de Platyhypnidium. De façon générale, les concentrations du magnésium (1 à 3 µg/g) et du potassium (2 à 4 µg/g) sont assez stables pour les deux genres. Quant au zinc, sa concentration est très faible chez les deux espèces et il ne semble pas être affecté par le polluant. Il est également possible qu'il y est une relation entre le sodium et le *magnésium ainsi qu'entre le calcium et le potassium chez Fontinalis lors du relargage.

RELATION ENTRE LA CONTAMINATION EN BPC DES GASTÉROPODES, DES MACROPHYTES ET DES SÉDIMENTS. C. Vanier[1], D. Planas[1], et M. Sylvestre[2]. [1]GEOTOP - Sciences de l'Environnement, Université du Québec à Montréal, PQ, H3C 3P8; [2]INRS - Santé, Pointe-Claire, QC.

Les écosystèmes aquatiques, et plus particulièrement leurs sédiments, sont les milieux récepteurs de la majorité des contaminants hydrophobes et peu volatiles tels les biphényles polychlorés (BPC), ce qui explique l'importance des sédiments comme source de BPC pour les organismes aquatiques. Les macrophytes enracinés colonisant des sédiments contaminés sont ainsi exposés à des concentrations très élevées en BPC. Ils sont de ce fait possiblement impliqués dans le transfert de BPC des sédiments vers le reste du réseau trophique, *via* les organismes brouteurs. Des sédiments (15 cm analysés en couches de 5 cm), des macrophytes (tiges et racines analysées séparément) et des gastéropodes épibenthiques ont été échantillonnés à trois stations du lac Saint-François (Fleuve Saint-Laurent, Canada). Les sédiments et les macrophytes (tiges et racines) présentent un gradiant décroissant de contamination entre les trois stations. Bien que les racines de macrophytes soient beaucoup plus contaminées que les tiges, les résultats préliminaires montrent une distribution des congénères de BPC similaire dans les trois compartiments à l'étude, suggérant un transfert de BPC des sédiments aux racines et leur translocation vers les tiges des macrophytes.

MARINE MICROSCALE ECOTOXICOLOGY: ASSESSMENT OF THE MICROTOX^R BIOASSAY FOR INCLUSION IN A TIERED HAZARD EVALUATION OF SEDIMENTS FROM HALIFAX HARBOUR, NOVA SCOTIA. N.H. Cook and P.G. Wells. School for Resource and Environmental Studies, Dalhousie University, 1312 Robie Street, Halifax, NS, B3H 3E2.

Subtidal sediments were collected from eight established stations in Halifax Harbour, N.S., in July and August, 1992, to initiate a study of sediment toxicity and hazards near the proposed location of a sewage treatment plant and outfall. A set of 110 samples was evaluated. Interstitial water from aerobic surface (0-2 cm) and anaerobic subsurface (2-4cm) sediments was analyzed by the Microtox^R 100% toxicity test protocol, while the sediment particles were analyzed using the Microtox^R direct contact sediment assay. Toxicity was expressed as 5, 15, and 30 min EC_{50}'s for the interstitial samples, and 5 min EC_{50}'s for the sediments, the endpoint being the inhibition of photoluminescence of the test organisms, *Photobacterium phosphoreum.* Interstitial water from the surface and subsurface sediments from all stations was usually non-toxic, when compared to a phenol standard. Surface sediments from most stations had low toxicity while subsurface sediments were always toxic, toxicity thresholds being compared with National Research Council marine analytical sediment reference materials, and a selection of natural sediments. Intra- and inter-sample variability in toxicity at different locations in relation to time, sediment depth, sediment properties, and types and concentrations of contaminants, was evaluated by multivariate and PCA techniques. An experiment with spiked sediments (PAH's, metals, and their mixtures) showed the ability of the solid-phase assay to discriminate between different concentrations of contaminated sediments, i.e. a dose-response relationship was demonstrated. This laboratory-field study has provided

important insights into the discriminatory power of the Microtox^{R} solid-phase assay, and the field sampling strategy necessary to deploy it most effectively as part of a tiered hazard assessment of contaminated harbour sediments.

BIOACCUMULATION POTENTIAL OF THE ZEBRA MUSSEL (*Dreissena polymorpha*) AND ITS DISTRIBUTION AS A FUNCTION OF A PCB GRADIENT.

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The zebra mussel, which has recently invaded the Great Lakes and the St. Lawrence River, constitutes an important link in the transfer of contaminants from the base (algae) to the top of the trophic web (ducks, fish, ...). Zebra mussels have a life span of between 3 to 5 years. Living attached to the bottom, it feeds by filtering suspended particles (seston). Their high filtration rate (up to 200 ml/hr) in addition to their high lipid content (10 to 25%) favor the accumulation of a wide range of liposoluble and hydrophobic contaminants such as the PCBs. The principal objective of this study is to determine the contamination of zebra mussels and their role in the bioaccumulation of PCBs as a function of a contamination gradient. Zebra mussels were sampled inside and outsite a macrophytes beds in a fluvial lake (St. François Lake, St. Lawrence River) downstream of a contaminated site. Preliminary results indicate that PCBs contamination does not affect zebra mussel community structure and density. Densities in the most contaminated station (823 mussels/m^2) were higher than those of the less contaminated stations. The total PCBs (the sum of 33 congeners) present in zebra mussel are around 3.7ppm for the most contamined sites and decrease to <0.4 ppm at the less contaminated sites. The concentration of PCBs are higher in sites without macrophytes versus a sites with macrophytes.Because the greater abundance and PCBs concentration of zebra mussel in the most contaminated sites, these organism represent and hasard fot its predators.

TOXICITY TESTING OF SUSPENDED SEDIMENTS FROM THE ATHABASCA RIVER.

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Microtox testing of suspended sediment extracts from the Athabasca River in the region of the oil sands deposits shows a significant toxic response. The suspended sediments were extracted with dichloromethane, solvent exchanged with methanol and tested directly using the luminescent bacterium *Photobacterium phosphoreum*. Results are compared with previous Athabasca River toxicity results (1990) and with mixed function oxygenase levels in fish from the area (1991). Data will be used in conjunction with a two-dimensional chemical fate model being developed for risk assessment of future oil sands developments.

BIOASSESSMENT OF SEDIMENT FROM TEN GREAT LAKES AREAS OF CONCERN UTILIZING *Daphnia magna* AND *Hyalella azteca*.

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The current study examined the bioavailability of pollutants from a number of sites in the Great Lakes, using *Daphnia magna* and *Hyalella azteca* as aquatic assessors of the toxic potential of contaminated sediments. The Areas of Concern where sediment collection was carried out included Toronto and Hamilton Harbours, the Cuyahoga, Maumee, Raisin, Detroit, Rouge, and Manistique Rivers, and Saginaw and Green Bays. Contaminants under investigation included heavy metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Bioassays utilizing *D. magna* were 13-day tests examining both mortality and sublethal effects, including reproductive potential. *H. azteca* were also utilized in 13-day bioassays, with number of survivors being the measured endpoint. Bioassays using sediment from Hamilton Harbour and Rouge River indicated extreme toxicity to both organisms, with moderate toxicity noted where these animals were assayed with sediment from specific sites on the Cuyahoga River and Trenton Channel of the Detroit River. Concurrently, assays were conducted with rainbow trout to examine the biochemical responses of fish exposed to contaminated sediment, specifically those reactions relating to the induction of enzymes involved in the mixed function oxidase (MFO) system. The sediment bioassays measured the activity of the enzyme 7-ethoxyresorufin(*o*)deethylase (EROD). Although, to date, there is no demonstration of a causal link between increased MFO activity and other biochemical changes or deleterious impact in fish, induction of ERODs occurred with exposure to the same sediments that caused toxicity to *Hyalella* and *Daphnia*.

BENTHIC POPULATIONS IN NEAR-SHORE SEDIMENTS OF THE ST. CLAIR RIVER; 1990-1995; INTERIM REPORT.

I.W.E. Harris. Hazcheck Enterprises, 1310 Hillcrest Drive, Sarnia, ON, N7S 2N4.

When completed, a six-year study will provide a profile of the benthic invertebrate population in the near-shore waters of the St. Clair River. Diversity and density are reported for eighteen sample sites on both the Canadian and U.S. side from Point Edward, ON, to Algonac, MI. At each sample site, three Petite Ponar grabs are taken once per year at a depth of two metres. The timing of sampling is scheduled to provide an estimate of the seasonal effect. Sediment characteristics are recorded as volume percent gravel (size >2 mm), sand (2 mm > size >0.2 mm), mud (size <0.2 mm) and vegetation. River flow rate is measured and total nitrogen and total phosphorous data are derived from the open literature. With increasing downstream distance, diversity and density of benthic populations increase toward asymptotes of 22 families/site and 5,000 total organisms/metre^2 respectively. Sites with high proportions of coarser sediment particles show lower diversity and density. A slight increase in diversity and density is evident as the sampling season advances. River flow rate and nutrient levels are not significantly variables. An archive of all specimens collected is being accumulated; labelled for sample site, sample date and invertebrate order, all preserved in 70%

isopropanol.

PENETRATION OF PESTICIDES IN PLASTIC USED FOR CONTAINER MANUFACTURE AND RECYCLING. G.M. O'Brien. Department of Environmental Biology, University of Guelph, Guelph, ON, NIG 2W1.

High density polyethylene, HDPE, is used extensively in the manufacturing of pesticide containers. These containers are not suitable for reuse and have traditionally been disposed of by incineration, landfilling and burying. In recent years, there has been an initiative to recycle these plastic containers into potentially useful products which include sign posts, fence posts and curb stops. However, there is measurable pesticide dislodgability and leachability from these products. Evidence suggests that pesticides, particularly solvent based formulations, can penetrate into the plastic matrix of containers during storage. Radiolabelled commercial grade formulations of 2,4-D isooctyl ester, atrazine, azinphos-methyl, sethoxydim, trifluralin and metolachlor, were used to examine penetration into HDPE and fluorinated HDPE containers. Plastic disks taken from containers were clamped to the top of crimp-cap septum vials containing the radiolabelled pesticides. Vials were inverted and incubated at three different temperatures and stored for various periods up to 24 months. At the end of a specified time period, the disks were removed from the vials, subjected to a triple rinse, and completely oxidized. Radioactivity from the plastic disks, the rinses and the formulations, were determined to assess the degree of pesticide penetration into the plastic. The objective of this experiment is to develop a methodology that can be used to determine formulation and container combinations which would minimize penetration. This would provide quality control for container recycling and aid in the estimation of environmental and human risk. Preliminary results will be presented.

ENVIRONMENTAL AND TOXICOLOGICAL SIGNIFICANCE OF THE COELUTION OF PCB CONGENERS 132 AND 153. A.J. Niimi[1] and H.B. Lee[2]. [1]Department of Fisheries and Oceans, Bayfield Institute, Canada Centre for Inland Waters, Burlington, ON, L7R 4A6; [2]Environment Canada, National Water Research Institute, Canada Centre for Inland Waters, Burlington, ON, L7R 4A6.

2,2',4,4'5,5'-Hexachlorobiphenyl (IUPAC No. 153) is one of the most common PCB congeners reported in various sample matrices. Chromatographic properties for PCB 153 suggest it may co-elute with 2,3,3',4,4'-pentachlorobiphenyl (PCB 105) and should co-elute with 2,2',3,3',4,6'-hexachlorobiphenyl (PCB 132). Separation and quantitation of PCB 105 may not be too difficult using more selective chromatographic conditions, or a mass selective detector. Separation of PCB 153 from 132 is more difficult, but can be achieved using nonconventional methods such as multidimensional chromatography. This study examined the relationship between congeners 132 and 153 by analyzing their concentrations in extracts from Great Lakes fish. Samples were analysed by gas chromatography equipped with a mass selective detector using a nonpolar column commonly used in routine PCB analyses, and a more polar column. The chromatograph using the nonpolar column showed one well-defined peak commonly been identified as PCB 153, and two peaks when the more polar column was used. Approximately 25% of the concentration generally reported as PCB 153, may be attributable to PCB 132. These results indicate most routine measurements reported for PCB 153 may actually represent PCBs 153+132, and should be reported accordingly. This coelution factor could influence toxicological assessment because of the differences in chlorine substitution patterns. PCB 132 is substituted at three *ortho-*, two *meta-* and one *para-* positions, while PCB 152 is substituted at two *ortho-*, two *meta-* and two *para-* positions. Toxicological studies have suggested (1) congeners with two *ortho-* substitutions are more toxic than those with three or four substitutions, (2) congeners with no substitution at adjacent *ortho-* and *meta-*, or *meta-* and *para-* positions are more amenable to metabolic hydroxylation, and (3) congeners substituted at both *para-* and at least two *meta-* postions may be more toxic. Both congeners differ in all criteria.

CANADIAN SEDIMENT QUALITY GUIDELINES FOR MERCURY. S.M. Lingard[1], K.A. Keenleyside[2], S.L. Smith[2] and C.L. Gaudet[2]. [1]Lingard Consulting, Ottawa, ON, K1K 2K6; [2]Evaluation and Interpretation Branch, Environment Canada, Ottawa, ON, K1A 0H3.

The Canadian Council of Ministers of the Environment (CCME) has recently developed a science-based protocol for deriving sediment quality guidelines for the protection of aquatic life. These guidelines provide nationally consistent benchmarks for evaluating sediment quality and are intended as decision support tools in protecting and sustaining aquatic ecosystems in Canada. They can be used as triggers for further investigation, including ecological risk assessment, and to support the development of site-specific objectives and remediation objectives. Interim sediment quality guidelines are being developed for mercury in freshwater and marine ecosystems following methods described in the formal protocol. Preliminary calculations indicate that interim marine and freshwater sediment quality guidelines for the protection of aquatic life will both be 0.14 mg/kg. Final recommended values are subject to change pending final approval by the Canadian Council of Ministers of the Environment.

A POTENTIAL METHOD FOR DERIVING SAFETY FACTORS FOR WATER QUALITY GUIDELINES WHICH PROTECT AQUATIC LIFE. H. Vandermeulen[1], D. MacDonald[2] and R.A. Kent[1]. [1]Environment Canada, Evaluation and Interpretation Branch, Ottawa, ON, K1A 0H3; [2]MacDonald Environmental Sciences Ltd., Ladysmith, BC, V0R 2E0.

Canada produces national environmental quality guidelines for toxic substances via a co-operative federal / provincial process sanctioned by the Canadian Council of Ministers of the Environment (CCME). The CCME has used standard safety factors in the past which are founded on limited scientific empirical evidence. A proposal will be presented on an empirical method for deriving safety factors which classifies toxic substances based upon mode of action. The authors believe that safety factors need to be generated based upon the level of protection which the final guidelines aim to uphold. The philosophical reason why an empirical rather than stochastic method has been chosen will be discussed. Elements of the safety factor derivation process will be described.

CRIER: A PRELIMINARY SCREENING TOOL FOR ANALYZING THE RELATIVE RISK OF MULTIPLE TOXICANTS IN AQUATIC ENVIRONMENTS. S. Brucher, P. Melanson and P.D. Anderson. Concordia's Ecotoxicology and Environmental Chemistry (CEEC) Unit, Concordia University, Montreal, QC, H3A 1M8.

A preliminary screening tool for analyzing the relative risk of toxic constituents of complex effluents to aquatic organisms has been developed. The computer-based tool, known by the acronym CRIER, accounts for a common set of environmental events and bioconcentration in deriving a tentative estimate of the availability of each of several chemicals to a target organism. This estimate is compared to a standard toxicological threshold concentration to give a risk factor of the respective chemicals. Each chemical is then indexed according to a ranking system that separates chemicals into high, moderate, low and no-apparent risk categories. Allowance is provided for the cumulative toxicological effects of similarly acting chemicals to which organism may be concurrently exposed. The model has been developed for either a pelagic or benthic target organism. More than 150 chemicals, including inorganics as well as organics can presently be assessed by the model. To show the utility of CRIER, case studies are presented based on certain multicontaminated scenarios in the St. Lawrence river and some of its tributaries.

INFLUENCE DE LA DURETÉ DE L'EAU SUR L'ACCUMULATION DU CADMIUM CHEZ *Fontinalis dalecarlica*. L. Pazdernik et S. Bourgoing. Département de chimie-biologie, Université du Québec à Trois-Rivières, Trois-Rivières, QC, G9A 5H7.

La présence de micropolluants en faibles quantités dans le milieu nécessite l'utilisation d'un indicateur capable de réfléter la qualité de l'eau. Lors d'étude "in situ", les caractéristiques de l'eau varient d'un endroit à l'autre et il importe de connaître la réaction de l'organisme sentinelle face à ces conditions changeantes; la dureté étant liée aux conditions locales et aux influences anthropiques. La toxicité d'un grand nombre de métaux lourds (Stumm et Morgan 1970, Alabaster et Lloyd 1982) est influencée par la dureté de l'eau. Or, l'objectif principal de cette expérimentation est de démontrer l'effet de la dureté de l'eau sur l'accumulation du cadmium et en second lieu d'évaluer le taux d'assimilation par une mousse aquatique. Les résultats démontrent que les ions Ca^{++} et Mg^{++} inhibe l'entrée du cadmium dans les mousses tel qu'en témoigne les facteurs de bioconcentration (FBC). Pour une concentration de 5 µg/L de Cd le FBC est de 20 000 en eau douce et de 16 500 en eau dure. Un effet de compétition entre les cations reliés à la dureté de l'eau et le toxique pourrait expliquer ces différences de contamination. Quant aux concentrations dans les mousses, elles sont respectivement de 20, 90 et 150 µg/g pour des concentrations de 1, 5 et 10 µg/L . Malgré ces différences entre l'eau dure

et l'eau douce, le genre Fontinalis demeure un excellent choix comme traceur de métaux puisqu'il atteint un plateau d'accumulation après trois jours d'exposition.

VARIATION DES MÉTAUX ESSENTIELS ET DES POLLUANTS CHEZ LE GASTÉROPODE *Viviparus georgianus*. L. Pazdernik, L. Tessier, S. Gélinas, B. Paris et G. Vaillancourt. Département de Chimie-Biologie, Université du Québec à Trois-Rivières, Trois-Rivières, QC, G9A 5H7.

Plusieurs métaux nécessaires à la vie des gastéropodes tels que Ca,Cu,Fe,Mg,Mn et Zn, sont trouvés dans l'environnement et dans ces espèces à des concentrations élevées. De plus, il y a d'autres métaux provenant de rejets industriels qui s'ajoutent à la diète des organismes aquatiques. Notre étude de l'accumulation et de l'élimination de deux métaux pollulants, (le cadmium et le mercure) pendant 60 jours démontrent des patrons physiologiques biphasique. Les gastéropodes accumulent ces métaux assez rapidement au début et ensuite plus lentement dans le deuxième phase qui se traduit par un plus grand facteur de bioconcentration. Par contre, peu d'études font l'objet de la bioaccumulation et de la synergie des métaux conventionnels. Puisque le magnésium est un cation bivalent intracellulaire détenant un rôle central dans plusieurs processus cellulaires, les travaux en parallel que nous avons effectués vise principalement l'étude de celui-ci. Dans un premier temps, une comparaison entre les concentrations de magnésium de la chaire des gastéropodes provenant de deux stations différentes dans le fleuve St-Laurent, a permi l'observation de concentrations nettement différentes chez les individus de classe d'âge 2 et 3 ans. Dans un deuxième temps, nos résultats démontrent une bonne corrélation entre l'âge des individus et la bioaccumulation de Ca,Cu,Mg,Mn et Zn. De plus, ces six métaux peuvent être statistiquement divisés en deux groupes (Ca,Cu,Fe et Mg,Mn,Zn). La différence entre ces deux groupes semble être liée au rôle métabolique spécifique chez le gastéropode.

MODÉLISATION MATHÉMATIQUE DE LA DISPERSION DES FLUORURES PAR RAPPORT AUX QUANTITÉS RETROUVÉES A PROXIMITÉ D'UNE ALUMINERIE. P. Francoeur et L. Pazdernik. Département de chimie-biologie, Université du Québec à Trois-Rivières, Trois-Rivières, QC, G9A 5H7.

Les procédés de fabrication de l'aluminium génèrent des fluorures qui sont en partie libérés dans l'atmosphère. La simulation par ordinateur de dispersion atmosphérique permet d'avoir une approximation des quantités de contaminants que l'on peut retrouver dans l'environnement à partir de diverses sources. Le logiciel BLP, spécialement conçu pour les alumineries, permet de déterminer les quantités de fluorures à partir des émissions de l'aluminerie. Dans un premier temps, une comparaison sera faite entre les quantités prédites par le BLP et celles captées à différentes stations près de l'aluminerie de Bécancour. Selon nos résultats, le capteur de la station située dans l'axe des vents dominants présente, pour les mois de juin à octobre 1992, des quantités de fluor allant de 12.3 à 23.3 $\mu g/m^3$. Les quantités prédites par le BLP allaient de 6.4 à 17.9 $\mu g/m^3$.

Dans un deuxième temps, une corrélation sera établie entre les quantités prédites par le BLP et celles retrouvées dans certaines espèces végétales. Finalement, nous prévoyons examiner la relation mathématique existante entre la quantité des émissions de fluorures et celles retrouvées dans les champs par des capteurs chimiques.

SURVEY OF ORGANOCHLORINES IN YUKON FISH. J.E. Eamer. Environmental Conservation Branch, Environment Canada, Box 6010, Whitehorse, YT, Y1A 5L7.

Fish collected from 1990 through 1993 from Yukon lakes were analysed for organochlorines. The main organochlorine contaminants detected were toxaphene, PCBs, and DDT and its metabolites. Of these, toxaphene is of most concern, as it is the most abundant and widespread. While levels are low in most fish from most lakes, toxaphene in high-lipid-content tissues from predator fish in two lakes have resulted in Health Canada consumption advisories. Related ongoing studies show that a major source of organochlorine contaminants in the Yukon is long range atmospheric transport. Past use and disposal of DDT and PCBs in the territory have undoubtedly contributed to the levels of these contaminants in some lakes. The very limited local historic use of toxaphene does not appear to have contributed to current toxaphene concentrations in Yukon fish. Hypotheses concerning variability in contaminant levels among locations are discussed. Initial surveys of lakes and rivers designed to assure Yukoners of the safety of fish for human consumption are near completion. Efforts are now being directed to collecting the baseline data needed to detect changes over time in levels of organochlorines in aquatic ecosystems. This project is directed by the Yukon Contaminants Committee, a committee of government, First Nation, environmental group and academic representatives, and is funded through the Arctic Environmental Strategy.

2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN EQUIVALENTS IN FISH TISSUE FROM THE ST-LAWRENCE RIVER. C. Brochu, G. Hamelin, D. Laliberté and S. Moore. Ministère de l'Environnement et de la Faune du Québec, Quebec, QC, H7C 2M7.

The aquatic environment has been known to be contaminated by complex mixtures of planar chlorinated hydrocarbons such as polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). Because the toxic effect produced by these components appears to be related through a common receptor mechanism of action, the potency of individual congener can be reported relative to the 2,3,7,8-TCDD (2,3,7,8-TCDD Equivalent). The concentration of 2,3,7,8-TCDD Equivalent in three fish species (brown trout, chinook salmon and eel) from the St-Lawrence river was determined from the concentration of 3 planar PCB (#77, #126, #169), 8 mono-ortho PCB, 2 di-ortho PCB and the 17 congeners of 2,3,7,8 PCDD/PCDF. Consensus PCB toxic equivalency factor (TEF) recently published by the WHO-European Centre for Environment and Health (1994) were used to evaluate the contribution of each group of congeners to the total 2,3,7,8-TCDD Equivalent. For all these species, the total 2,3,7,8-TCDD Equivalent concentration is over the acceptable level proposed by Health and cladocerans, copepods, amphipods, and rotifers. Artificial aquatic microcosms consisting of a simple community structure, including fish, algae, macrophytes and invertebrates, were utilized. Seven doses of creosote (ranging from 10 ppm to 1000 ppm) and three controls were used. Dosing was accomplished by adding creosote to the microcosms and allowing it to partition to the various compartments within the microcosms. As well, 1 to 8 creosote coated log pilings were added to several microcosms, simulating aquatic exposure to preserved log pilings. The zooplankton at the various dose levels were enumerated.

MIXED-FUNCTION OXYGENASE (MFO) ENZYMES AS A TOOL FOR ECOTOXYCOLOGICAL FIELD STUDIES ON FISH OF THE ST.LAWRENCE RIVER.

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The Mixed-Function Oxygenase (MFO) enzyme system plays a central role in the metabolism of a variety of xenobiotic and endogenous compounds. MFO activity is considered as one of the more advanced tools available for the assessment of early effects of toxic substances in natural fish populations. This study reports on the variability in MFO activity for 3 different fish species from different sampling sites in the St.Lawrence River. MFO activity was measured by two different enzymes: Ethoxyresorufin-O-deethylase (EROD) and Aryl hydrocarbon hydroxylase (AHH). In a first series of experiments, a significant difference of MFO activity was observed among four populations of White Sucker (*Catostomus commersoni*) coming from four tributaries of St.Lawrence River. The highest MFO values were recorded for fish caught at the Yamaska station which has the type of pollution that induce MFO. In the second experiment, MFO activity was measured on 2 fish species collected at 3 sites in lake Saint-Louis (along the St. Lawrence River) on 2 sampling periods. The 2 species were Yellow Perch (*Perca flavescens*) and Northern Pike (*Esox lucius*). Among-stations difference was significative for the Northern Pike during one sampling period only. MFO activity was highest at the Pointe Thibaudeau station. This station is located within a heavy industrial pollution area. Results indicate that MFO varies with individual fish, leading to high within-station variability. Consequently, we suggest that the MFO responses may be useful for delineating potential areas of high pollutant impact.

ÉVALUATION DU POTENTIEL DE L'HIRONDELLE BICOLORE COMME ESPÈCE BIOINDICATRICE DE LA CONTAMINATION DU FLEUVE ST-LAURENT.

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Le fleuve Saint-Laurent est très vulnérable à la contamination par les substances toxiques. Un des objectifs du Plan d'Action Saint-Laurent est la réduction de 90% de la charge toxique des rejets liquides des principales usines situées le long du fleuve. Le succès des actions entreprises pour nettoyer le fleuve est évalué entre autres au moyen d'un réseau de surveillance de la contamination des chaînes alimentaires à l'aide d'espèces bioindicatrices. Une des espèces envisagées est l'Hirondelle bicolore (*Tachycineta bicolor*). Des nichoirs ont été installés en aval des effluents de 10 usines parmi les plus préoccupantes le long du fleuve Saint-Laurent, de même qu'à deux stations témoins. Des oeufs, des jeunes et des adultes ont été récoltés pour des fins d'analyses afin de détecter la présence de pesticides organochlorés, de congénères de BPC et de mercure. Les résultats indiquent que les Hirondelles bicolores accumulent du mercure, du DDE et des BPC dans leurs tissus. Des différences sont observées entre les oeufs, les jeunes et les adultes, de même qu'entre les stations.

NONTARGET TOXICITY OF ANTIMYCIN A APPLIED TO MESOCOSMS FOR REMOVAL OF ENDEMIC FISH. K. Liber, S.L. O'Halloran, K.L. Schmude and T.D. Corry. Lake Superior Research Institute, University of Wisconsin-Superior, Superior, WI, 54880.

The fish toxicant antimycin A (Fintrol®) was applied to 18 mesocosms at 7.5 µg/L to remove endemic fish prior to initiation of a full-scale mesocosm experiment with a different chemical. Following removal of endemic fish, mesocosms would be restocked with different fish of known numbers. In addition to target fish populations, nontarget invertebrate populations were monitored within six mesocosms both before and after application of antimycin. Caged fish and trapping success studies indicated a high degree of success in removal of endemic fish. Caged endemic fish displayed 100% mortality while trapping success indicated that endemic fish abundances were reduced by 96%. Of the five major invertebrate taxa monitored (Cladocera, Copepoda, Ostracoda, Rotifera, Chironomidae), Cladocera and Copepoda displayed the greatest sensitivity to antimycin. Cladocera abundances were reduced by 76% seven days after application, but returned to pre-application levels within 21 days. Juvenile and adult Copepoda abundances were reduced by 51% and 67% seven days after application and returned to pre-application abundances within 14 and 21 days, respectively. Ostracoda and Rotifera abundances appeared unaffected by the antimycin application. Effects on Chironomidae were difficult to interpret due to a seasonal decline in population abundances, but effects appeared to be minor. It was concluded that antimycin-treated mesocosms had recovered within 28 days and were then ready for initiation of the actual mesocosm experiment.

USE OF MICROCOSMS TO VALIDATE AN ECOTOXICOLOGICAL RISK ASSESSMENT PROCEDURE. P. Takacs[1], R. Robinson[1], K.R. Solomon[1] and N.K. Kaushik[2]. [1]Canadian Network of Toxicology Centres (CNTC), University of Guelph, Guelph, ON, N1G 1Y4; [2]Department of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1.

In order to protect the environment from the effects of hazardous compounds a probabilistic risk assessment procedure may be used. Such a procedure suggests environmental concentrations of toxicants that will protect a certain percentage of the species present, eg. 95%. The present project focuses on the validation of a probabilistic risk assessment procedure using microcosms. These artificial pools were dosed with 5 different concentrations (0.01, 0.1, 1, 10, 200 ug/L) of the organophosphorous insecticide Azinphos methyl (Guthion). The range of test concentrations encompasses a sub-chronic to acute toxicity level. The microcosms, each 12m^3 in volume, contained: sediment placed in trays that cover 50% of the area, several pots of the macrophyte *Myriophyllum*, *Lemna*, periphyton, and fish were also among the test organisms. A pretreatment period provided time to minimize variability among ponds with circulation of water from the supply pond, as well as natural colonization of the microcosms. Treatment with Azinphos methyl was in the form of a solution of an emulsifiable concentrate poured into the ponds. Monitoring was conducted on parameters including community structure, function and water residues.

APPLICATION OF THE MICROTOX SOLID-PHASE TEST TO MONITOR THE EFFECTIVENESS OF BIOREMEDIATION STRATEGIES. K. Lee[1], R. Siron[2], G.H. Tremblay[1] and J. Lavoie[1]. [1]Maurice Lamontagne Institute, Department of Fisheries and Oceans, P.O. Box 1000, Mont-Joli, QC, G5H 3Z4; [2]Institut National de la Recherche Scientifique (INRS-Océanologie), Rimouski, QC, G5L 3A1.

INTRODUCTION

Microbiological studies in the laboratory, experimental field trials, and clean-up operations following actual marine oil spill incidents have conclusively demonstrated the effectiveness of a bioremediation strategy based on the enhancement of oil-biodegradation rates by nutrient addition (Lee and Levy, 1991). However, some environmentalists have expressed their concern of the net benefit of this bioremediation strategy because of: (1) the production of toxic metabolic byproducts associated with an accelerated oil biodegradation process; and (2) the ineffectiveness of natural metabolic processes to degrade the most toxic components within residual oils. The objective of this study was to test the suitability of Microtox® bioassay for measuring the toxicity of oiled marine sediments and to evaluate its use as a tool to monitor bioremediation treatment efficacy.

EXPERIMENTAL DESIGN

Initial tests were focused on the sensitivity of the Microtox® Solid-Phase Test (SPT) procedure to measure the toxicity of oiled marine sediments. Both the basic Solid-Phase Test (SPT) and the Large Sample Procedure (LSP) were evaluated using a Microtox® model 500 Analyzer (*Microbics Co.*, Carlsbad, USA). Data collection and reduction were made using version 7.0 of the Microtox® software, to calculate the Effective Concentration that induces a 50% reduction (EC_{50}) of bioluminescence of the test marine bacterium *Photobacterium phosphoreum* relative to a control population. These Solid-Phase Test protocols are based on the inhibitory effect induced when the test organisms are in contact with a suspension of toxic sediment (0.3g/3mL in the basic SPT; 7g/35mL in the LSP). EC_{50} values derived from dose-response curves ($R > 0.97$) were expressed in ppm of wet sediment.

An experimental field study was conducted in the inter-tidal zone of a low-energy beach located on the Atlantic coast of Canada, to assess the effectiveness of nutrient enrichment treatments to reduce the toxicity of residual oil stranded within sediments. On Day 0 (August 6, 1992), replicate *in situ* sediment enclosures were filled with 7L of sieved wet sand. Oil was added to selected enclosures at a concentration of 3% (w/v). A commercial organic fertilizer formulation (fish bone meal - N:P:K ratio: 6-10-1) was added to selected experimental enclosures (1730 g/L wet sediment) on Day 12 and subsequently after each sampling period (to maintain optimal nutrient concentrations). The experiment was terminated after Day 123 due to the onset of adverse winter conditions.

RESULTS

Results of preliminary assays on sediments contaminated with known concentrations of Venture Condensate showed a direct increase in sediment toxicity (i.e. an EC_{50} decrease) with concentration (Fig. 1). Toxicity measurements on uncontaminated sediments indicated that the Microtox® response was entirely due to the oil (no inherent sediment toxicity). There was no significant difference in toxicity results obtained when using either the Microtox® SPT standard protocol or the Large Sample Procedure (Figure 1). The residual toxicity of contaminated sediments from the field experiment was thus measured by the standard SPT procedure.

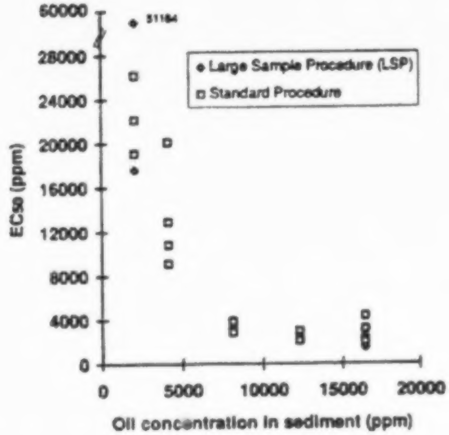

Figure 1

Change in sediment toxicity (EC_{50}, expressed in ppm wet sediment) as a function of Venture Condensate concentration. The two Microtox Solid-Phase Test (SPT) procedures were tested on standard sediment.

The efficacy of the fertilizer formulation to enhance the natural biodegradation rates of oil stranded within sediments was assessed by monitoring changes in the residual oil concentration and composition over a 123 day period. The Microtox® data (EC_{50}) showed that oil toxicity was reduced over time by natural procõesses including physical removal of the oil from the sediments, degradation, and evaporation. In terms of bioremediation by nutrient enrichment, periodic applications of fish bone meal fertilizers, during this experiment, failed to reduce sediment toxicity (Fig. 2). This unexpected failure, evident in both chemical (Fig. 3) and microbiological measurements (Lee et al. 1995), was due to: (1) selective growth of different bacterial populations or a diauxic response attributed to preferential use of the organic nutrient over the residual oil; and (2) production of toxic compounds, such as ammonia, from the metabolism of the organic fertilizer (Fig. 4).

CONCLUSIONS

The observed Microtox® response to oiled marine sediments confirms the sensitivity of this micro-bioassay (Siron and Pelletier 1994) and adds support for its potential use as a tool to monitor bioremediation treatment efficacy. Results of this experiment point out that while bioremediation is the most environmentally friendly of the new oil spill countermeasures, it should not be used without adequate knowledge of the available products and the environment. Furthermore, operational guidelines for its application should include ecotoxicological monitoring programs using oil-sensitive micro-bioassays.

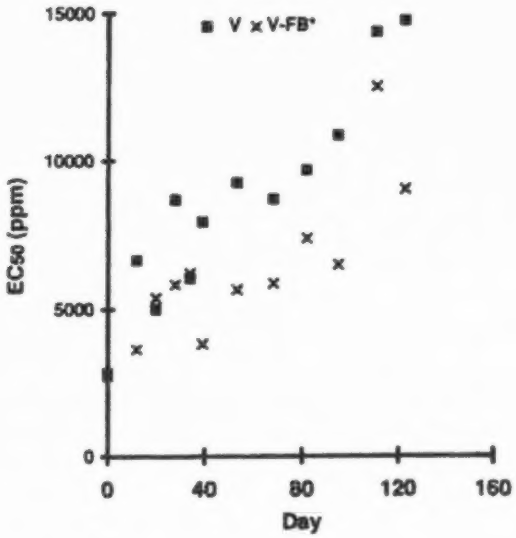

Figure 2

Change in the toxicity of oiled-sediment (EC_{50}, expressed in ppm wet sediment) in response to fertilizer applications. (V = Venture Condensate; V-FB* = Venture Condensate + Fish Bone Meal Fertilizer).

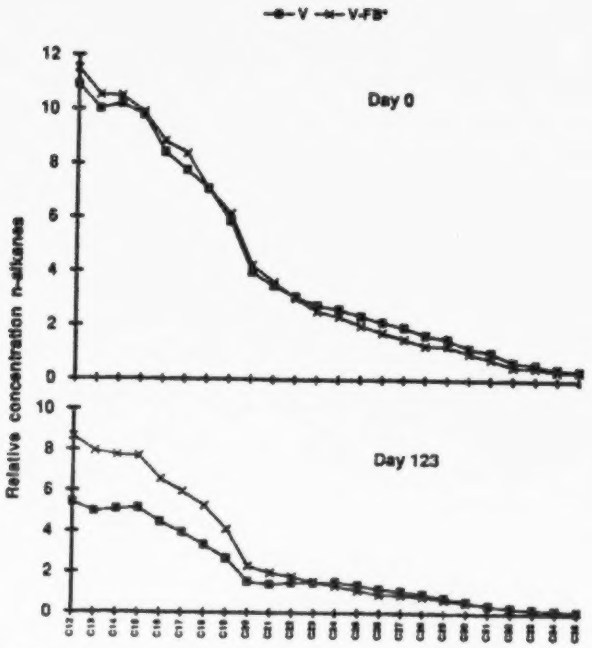

Figure 3

Time-series change in the relative concentration of *n*-alkanes (numbered by chain length) in response to nutrient treatment. (V = Venture Condensate; V-FB* = Venture Condensate + Fish Bone Meal Fertilizer).

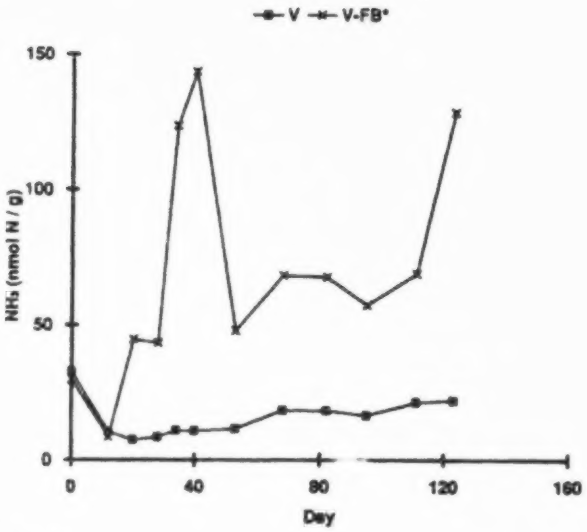

Figure 4

Ammonia release in response to tidal exposure. (V = Venture Condensate; V-FB* = Venture Condensate + Fish Bone Meal Fertilizer).

REFERENCES

Lee, K., and E.M. Levy. 1991. Bioremediation: Waxy crude oils stranded on low-energy shorelines, p.541-548. *In*: Proceedings of the 1991 International Oil Spill Conference, American Petroleum Institute, Washington, DC.

Lee, K., G.H. Tremblay and S.E. Cobanli. 1995. Bioremediation of oiled-beach sediments: Assessment of inorganic and organic fertilizers. *In*: Proceedings of the 1995 International Oil Spill Conference, American Petroleum Institute, Washington, DC. (In Press).

Siron R., and Pelletier É. 1994. Toxicity assessment of oil components and an oil treating agent using the *Photobacterium phosphoreum* bioassay, p. 164-178. *In*: Proc. 20th Annual Aquatic Toxicity Workshop, Can. Tech. Rep. Fish. Aquat. Sci., No. 1989.

BEST STUDENT PAPERS AWARDS/
PRIX POUR LES MEILLEURS EXPOSÉS PAR DES ÉTUDIANTS

BEST PLATFORM PRESENTATION

Extractable organochlorines (EOCls) in fish exposed
to bleached kraft mill effluent.

Helga E. Sonnenberg

Department of Environmental Biology, University of Guelph

BEST POSTER PRESENTATION

A preservable staining procedure to detect toxicant impacts on
algae (*Selenastrum capricornutum*) using flow cytometry.

Marvin J. Faber

Department of Environmental Biology, University of Guelph

A number of participants at the Workshop judged the student paper and poster presentations anonymously. The Workshop organizers wishes to thank these individuals for their time and effort, and their dedication to improving environmental toxicology education in Canada.

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ADDENDUM/ADDENDA

These contributions were presented at the 20th Annual Aquatic Toxicity Workshop held October 17-21, 1993, in Quebec City, Quebec, but were inadvertently omitted from the Proceedings of that Workshop.

DEVELOPMENT AND APPLICATION OF MICROSCALE BIOASSAYS IN AQUATIC ECOTOXICOLOGY. K. Lee[1], C. Blaise[2] and P.G. Wells[3]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, QC, G5H 3Z4; [2]Environment Canada, Centre Saint-Laurent, 1001 Pierre Dupuy, Longueuil, QC, J4K 1A1; [3]Dalhousie University, School for Resource and Environmental Studies, 1312 Robie Street, Halifax, NS, B3H 3E2.

Interest in the development and application of microscale bioassays (microbiotests) has increased dramatically in the field of aquatic ecotoxicology over the past 30 years. Broadly defined, these tests involve the exposure of acellular, unicellular or small multicellular organisms to a liquid or sediment sample in order to measure a specific effect (Blaise, 1991). Despite numerous studies, there is little evidence that the environmental impact of toxicants can be predicted accurately with a single species bioassay test. Future efforts at predicting pollutant impact should be directed towards the development of multi-trophic level, impact assessment tests. Hence, a test battery approach using a number of microbiotests is rapidly becoming the standard ecotoxicological test protocol for environmental impact assessment.

Both simple and cost-effective, microbiotests conducted with bacteria, micro-algae, protozoans, small invertebrates and their life stages, and eucaryotic cell-cultures can be routinely used in the laboratory to rank and screen potentially toxic chemicals. In addition, these tests show great promise for environmental impact assessments, since they enable the rapid detection of toxic compounds or constitutents (both organic and inorganic) in complex liquid/sediment samples. New developments and potential diagnostic applications of microbiotests based on advances in ecotoxicology, aquaculture, microbial ecology, genetics, biochemistry and biotechnology were presented and discussed during the current 1993 workshop session.

The session was organized with a review of microbiotests, followed by presentations on specific approaches representing different trophic levels from bacteria to fish. The final two papers described the evaluation and applications of multi-trophic level tests. The following highlights were made by the speakers:

(1). Marine microscale tests were reviewed by Wells, with a special emphasis on novel techniques, instruments and biological approaches. This presentation on the progress achieved in the development of microscale tests included a review of some of the major taxonomic groups (bacteria, microalgae, annelids, rotiferans, crustaceans and echinoderms) in use. Future research needs were identified; they include additional taxonomic clarification of some key groups (polychaetes, amphipods, copepods), the elucidation of the basis of the differential sensitivities observed across life stages and between species and phyla; and the incorporation of new molecular toxicology techniques.

(2). Siron described the use of the Microtox® test to evaluate the toxicity of component mixtures (water-soluble fraction) and pure compounds (aromatic and polyaromatic hydrocarbons) of a crude oil in seawater. The Microtox® test, based on the bioluminescence inhibition of the marine bacterium *Photobacterium phosphoreum*, was sensitive for this purpose. Subsequent mesocosm studies demonstrated the successful application of the test protocol to assess the toxicity of both crude oil and/or oil-treating agents.

(3). Legault described Environment Canada's use of the SOS Chromotest® under the St. Lawrence Action Plan to determine the genotoxicity of industrial samples. This microbiotest was found to be sensitive enough for the detection of genotoxic activity in neat industrial effluent samples. Legault also described the optimization of the SOS Chromotest® procedure to eliminate chemical interferences on the measurement of enzyme substrate reactions.

(4). There is considerable value to using indigenous bacteria for microbiotests as it brings ecological relevance to microscale assays. Lee described the development and evaluation of a toxicity test based on the impact of contaminants on a primary metabolic process the induction of microbial exoenzyme activity. The metabolic response of indigenous microbial species to inorganic and organic contaminants in both sediments and water was evaluated. This approach augments the use of several single species tests together, as this assay evaluates the response of the whole microbial community - a true multi-species test. Results of the test protocol showed significant agreement with some current single species tests (eg. Microtox® solid-phase test).

(5). Based on the results of field and laboratory studies on freshwater phytoplankton, Nalewajko reminded us of the very important fact that physiological strains exist within each species. Using experimental data, she illustrated changes in toxicity attributed to species specific responses, physiological strains, and the influence of physiological state of the organisms under study. Nalewajko stressed the ecological significance of using natural assemblages of species to examine community structure responses. The presentation emphasized the need for a battery of tests (even within the same trophic level), the importance of test selection based on the specific contaminant and field situations, and the need for additional studies on the identification of sublethal test end-points in phytoplankton.

(6). An overview on the use of microalgae for toxicity testing in Environment Canada's Longueuil lab was made by Blaise. The major applications of the microalgal toxicity tests since 1982 were described. One microalgal test has been well validated, standardized, and developed into an Environment Canada toxicity test protocol. Blaise described the development of algal assays into simple routine microplate tests by taking advantage of new developments in lab culture and instrumental technologies.

(7). The potential use of an amphibian newt (*Pleurodeles waltl*) micronuclei erythrocyte test was presented by Ferrier. This routine "in vivo" test was validated as a tool for examining genotoxic substances in water samples and industrial effluents. Results showed that this test has its place in a tiered strategy for testing the genotoxicity of wastewaters.

(8). A promising behavioural assay with a ciliated protozoan was described by Gilron. Based on their extensive ecological roles, protozoa should be considered more frequently in aquatic toxicity tests; they are an important food source for higher trophic level organisms, and act as major recyclers and mineralizers of organic material in aquatic ecosystems. While other ciliate bioassays have been developed, based on traditional indicators of toxicity (eg. growth and mortality), this behavioural microbiotest under development and validation is based on the measurement of a behaviour preference-avoidance response in a relatively simple apparatus.

(9). Riebel described test-species selection criteria and collection methods for some indigenous arctic marine invertebrates (mysids, amphipods, urchins). Results of preliminary microbiotests on the toxic responses of arctic invertebrate species to contaminants in both liquid and sediment were described.

(10). A simple and cost-efficient assay with Japanese medaka eggs was described in the presentation by Goudey. Study results showed that this assay, used extensively in Japan, can be used to examine sediments and water matrices. Different endpoints for toxicity (mortality, delayed development, and embryonic lesions) could be measured, and the variety of lesions were particularly well described.

(11). The results of a comparative study evaluating a large multi-species suite of standardized protocols using bacteria (*Photobacterium phosphoreum, Escherichia coli*), invertebrates (*Daphnia magna*) and fish (*Pimephales promelas*) were presented by Greene. Comparative results of the suite of toxicity tests after the evaluation of more than 200 elutriate test solutions (prepared from soil or sediment, ground water and surface water) support the multispecies test approach.

(12). MacDonald described the recent application and interpretation of a battery of marine microscale tests at the Saint John Harbour ocean disposal site. This study compared the relative sensitivity of the pore water and solid phase Microtox® tests (*Photobacterium phosphoreum*), two amphipod (*Amphiporeia virginiana; Rhepoxynius abronius*) survival tests, the sea urchin (*Lytechinus pictus*) fertilization test and the indigenous bacterial exoenzyme test. The highest sensitivity was observed in the Microtox® solid phase assay, the indigenous bacteria exoenzyme assay, and the sea urchin fertilization assay. Results of the study were used to formulate criteria for the acceptability of harbor sediments for disposal under the Canadian Environmental Protection Act, Part 6.

The recognized need for multi-trophic level ecotoxicity assessments and for sensitive young life-stage assays has driven the recent increase in development and use of microbiotests (Wells 1991, Lee et al. 1990). Technical papers presented at the workshop on the species specific tests covered a wide range of trophic levels. Overall, there was an emphasis on improving the simplicity, cost-effectiveness and reliability of microbiotests to enable the large scale screening of xenobiotics (singularly or in mixtures) in the environment. The presentations on the application of multi-trophic level tests emphasized the importance of choosing test protocols to fit the environmental conditions at the specific site in question.

The need for more fundamental research to define operational guidelines for ecotoxicological test programs and to understand the basis behind the relative sensitivities of the common tests was emphasized during the session. Inconsistencies in conclusions in papers within the session on the sensitivities of particular assays were apparent. To fully understand the reasons for the differences, more interaction between research groups to assess the toxicity of similar samples using different test protocols and intercalibration exercises using the same protocols are needed.

Microscale bioassays have become an essential and often routine part of ecotoxicological studies. They have now been adapted as standard tools in our national environmental monitoring programs for the diagnoses of anthropogenic stresses on the environment. These assays offer many advantages and opportunities for problem identification, research, assessment, monitoring, and pollution regulation. The development and application of microbiotests will be the focus of much attention during the 1990's in our continued effort to protect and conserve aquatic ecosystem health.

REFERENCES

Blaise, C. 1991. Microbiotests in aquatic ecotoxicology: characteristics, utility, and prospects. Environ. Toxicol. Water Qual. 6: 145-155.

Lee, K., K.L. Tay, C.N. Ewing and E.M. Levy. 1990. Toxicity and environmental impact assessment tests based on the activity of indigenous bacteria. Ocean Dumping Report 4, Environment Canada. 138 p.

Wells, P.G. 1991. Microscale marine toxicity tests – an introduction, p. 320-322. *In* Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, B.C. P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuk and W. Knapp, (ed.). Can. Tech. Rep. Fish. Aquat. Sci. No. 1774 (Vol. 1).

DEVELOPMENT OF ENVIRONMENTAL IMPACT ASSESSMENT TESTS BASED ON THE EXOENZYME ACTIVITY OF INDIGENOUS BACTERIA. K. Lee[1], R. Larocque[1] and K.L. Tay[2]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000. Mont-Joli, QC, G5H 3Z4; [2]Environment Canada, Conservation and Protection, 45 Alderney Drive, Dartmouth, NS, B2Y 2N6.

Marine sediments act as effective sinks for contaminants. While chemical analyses of sediments from a given area reflect the degree and nature of contamination, such chemical data provide little or no evidence on the biological consequences of the contamination. Since both chemical and biological processes in sediments (including organic matter degradation, nutrient regeneration and trace metal transformation) are predominantly mediated by the activity of microorganisms, effects on the microbial community and its activity in sediments should be considered an essential part of any environmental impact assessment. Natural bacterial communities can be used for monitoring changes in the marine environment since they are ubiquitous and respond rapidly to alterations in their environmental conditions. To gain complete understanding of the environmental effects of contaminants, effects must be examined at the ecosystem level, not just the population and community level. The focus should be on primary metabolic processes since a disturbance of these processes will have significant ramifications throughout the ecosystem. Furthermore, to obtain ecologically valid evidence on the potential damage of contaminants, tests should be conducted with indigenous biota in water and sediment from the specific sites in question.

The vital importance of enzymes at both the cellular and ecosystem levels is universally recognized. In bacteria for example, high molecular weight compounds must be cleaved outside the cell wall by exoenzymes before uptake can occur. Since the toxicity of most pollutants is attributed to enzyme inhibition, bioassays based on enzyme activity may be more sensitive and population level (LD_{50}) tests, and more rapid, cost-effective and reliable than measurements of whole organism activity. While direct measurement of enzyme activity in sediments may give a better indication of their ability to degrade organic pollutants, the measurement of enzyme induction rates in organisms, stressed by pollution, may be the most ecologically relevant test. Biochemical measurements of changes in bacterial exoenzyme activity, a primary metabolic pathway, in natural sediments could be used to: (1) monitor conditions at sites to ascertain conditions before and after industrial; (2) pre-screen wastes for toxicity and to determine allowable limits for oceanic disposal; and (3) determine the size of benthic impact zones; and 4) monitor the recovery of sites following impact.

The mineralization of organic matter in sediments is a key component of the carbon and nutrient cycles in the aquatic environment. Since bacteria are unable to assimilate large macromolecules directly, the hydrolysis of polymeric organic compounds (eg. proteins, polysaccharides, nucleic acids and humic materials) by specific inducible exoenzymes, is generally acknowledged as the first and rate-limiting step in mineralization. Because of recent improvements in biochemical techniques, the activity of many exoenzymes (eg. glucosidase, phosphatase and protease) in natural waters and sediments can now be measured by using specific substrates conjugated with the fluorophore, methylumbelliferone (MUF) or methyl coumarinyl amide (MCA). The high fluorescence of the MUF or MCA released following the hydrolysis of these substrates allows measurement of enzymatic activity with short incubation times, at ambient temperatures, and at substrate concentrations similar to those found in nature.

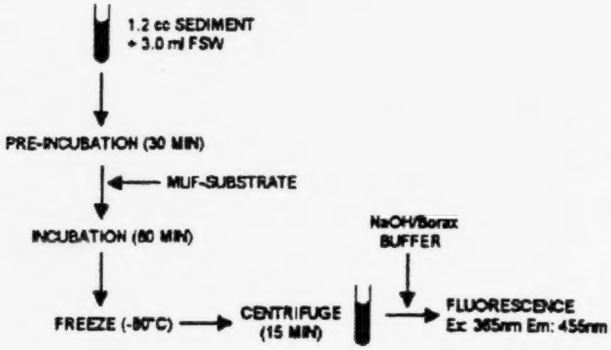

Fig. 1. Analytical methodology for the measurement of microbial exoenzyme activity in sediments.

Experiments have been conducted to establish a rapid low-cost standard protocol for the measurement of exoenzyme activity in marine sediments using Methylumbelliferone (MUF) and methylcoumarinylamine MCA) based fluorogenic substrates (Fig. 1).

MUF-β-glucoside, MUF-phosphate, MCA-leucine and MCA-alanine were found to possess high activity rates within marine sediments. The sensitivity of the microbial exoenzyme systems to selected contaminants (e.g. water-soluble fraction of Scotian Shelf condensate) was assessed in the laboratory using experimental "aquaria-type" enclosures. Preliminary field studies on its potential use for environmental impact assessment have been conducted with a wide range of contaminants associated with dredge spoils disposal (Fig. 2).

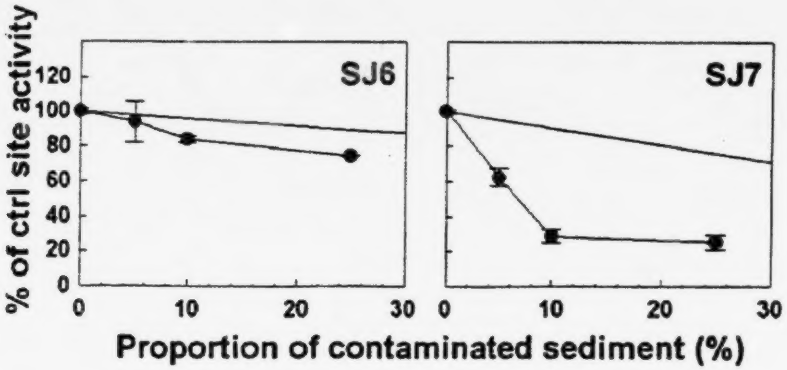

Fig. 2. Microbial bioassay based on the induction of exoenzyme activity. Representative test results showing the effect of relatively "unpolluted" sediment (SJ6) and contaminated sediment (SJ7) from Saint John Harbour, NB. on exoenzyme activity in control site sediments. Straight line represents the effect of dilution by experimental sediments on control site sediments.

With further development and validation, this procedure may provide a rapid means of determining the status of the benthic environment (results obtained in a matter of hours), it can be used as an early warning system that allows remedial action to be taken before permanent damage to living resources occurs.

OIL SPILL BIOREMEDIATION STUDIES IN LOW-ENERGY SHORELINE ENVIRONMENTS. K. Lee and G.H. Tremblay. Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, QC, G5H 3Z4.

Nutrient-enhanced bioremediation is a procedure in which the biodegradation rate of pollutants is enhanced by providing indigenous microorganisms with the "limiting" nutrient.

It is now recognized as one of the most promising technologies for the cleanup of oil-contaminated shorelines (Lee and Levy 1989).

The Department of Fisheries and Oceans has research program to: (1) study the natural degradation rates of petroleum hydrocarbons spilled or stranded in shoreline environments, and (2) to test the efficacy of selected nutrient formulations to enhance the natural rate of biodegradation. These bioremediation studies in low-energy shoreline environments (sand beaches and salt-marshes) have included both waxy-crude oils (Hibernia and Terra Nova) and condensate (Scotian Shelf) recovered from exploratory wells located on the east coast of Canada.

Initial laboratory studies conclusively demonstrated that bioremediation rates of Scotian Shelf Condensate spilled in sandy sediments could be enhanced by the addition of an oleophilic nutrient formulation (developed to maintain nutrients at the oil-water interface where biodegradation occurs). However, corresponding *in situ* studies within a sandy beach environment were hindered by the physical loss of the oleophilic nutrient from the sediments in a matter of days (Lee and Levy 1987). To maintain optimal nutrient concentrations, the experiments were repeated with periodic nutrient additions. Both organic nutrient formulations (a commercial oleophilic product) and water-soluble inorganic fertilizers were evaluated (Lee and Levy 1993, 1992, 1989). Quantitative chemical analysis (GC-FID) and ^{14}C-hexadecane radiotracer studies indicated that inorganic nutrient additions (eg. slow-release agricultural fertilizer) were just as, or more efficient than, the addition of the oleophilic product due to a diauxic growth response. In general, nutrient additions stimulated microbial activity and prolonged the seasonal period of oil degradation. Recent studies using gas chromatography-mass spectroscopy have conclusively confirmed an increase in the depletion rates of normal alkanes, and polyaromatic hydrocarbons (including toxic/carcinogenic fractions) relative to conservative markers (hopanes) in the residual oil following nutrient enrichment.

Like all other oil-spill countermeasures, bioremediation has its operational limitations. It is highly unlikely that a bioremediation process will ever degrade 100 percent of the components in a crude oil. The residual components composed of high molecular weight compounds including most of the asphaltenes and resins, as well as refractory molecules of the saturate and aromatic compounds will remain. The biodegradation process requires time. For example, in most of our experiments, the biodegradation of alkanes did not become obvious until after 10 to 20 days. Our studies indicate that nutrient-enhanced bioremediation should be considered as one of the many oil-spill countermeasure technologies, including the option of no treatment (Lee and Levy 1991). Before it becomes a practical oil-spill response technology, further research and development programs are required to identify its operational limitations (under what conditions does it work), and to improve application methodologies (type of fertilizer, frequency of application, etc).

REFERENCES

Lee, K., and E.M. Levy. 1987. Enhanced biodegradation of a light crude oil in sandy beaches, p. 411-416. Proceedings of the 1987 Oil Spill Conference, American Petroleum Institute.

Lee, K., and E.M. Levy. 1989. Biodegradation of petroleum in the marine environment and its enhancement, p. 217-243. *In*: Aquatic Toxicology and Water Quality Management, J.O. Nriagu and J.S.S. Lakshminarayana (ed.), John Wiley and Sons, Inc., NY.

Lee, K., and E.M. Levy. 1989. The enhanced biodegradation of condensate and crude oil on beaches of Atlantic Canada, p. 479-486. Proceedings 1989 Oil Spill Conference, American Petroleum Institute.

Lee, K., and E.M. Levy. 1991. Bioremediation: Waxy crude oils stranded on low-energy shorelines, p. 41-549. Proceedings of the 1991 International Oil Spill Conference, American Petroleum Institute.

Lee, K., and E.M. Levy. 1992. Microbial degradation of petroleum in an intertidal beach environment: In situ sediment enclosure studies, p. 140-156. *In*: Marine Ecosystem Enclosed Experiments: Proceedings of a symposium held in Beijing, People's Republic of China, May 9 to 14, 1987. C.S. Wong and P.J. Harrison (ed.), International Development Research Centre (IDRC), Ottawa.

Lee, K., G.H. Tremblay and E.M. Levy. 1993. Bioremediation: Application of slow-release fertilizers on low-energy shorelines, p. 449-454. Proceedings of the 1993 International Oil Spill Conference, American Petroleum Institute.

Strain, P.M. 1989. The persistence and mobility of a light crude oil in a sandy beach. Mar. Environ. Res. 19: 49-76.

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER

The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to various libraries, government departments and other agencies that are listed on the Department of Fisheries and Oceans technical reports mailing list. Copies of most Proceedings, as photocopies or fiche, are available for a charge from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250). Their catalog numbers (MLCN) are listed below where applicable.

Proceedings of the Twentieth Annual Aquatic Workshop: October 17-21, 1993, Quebec City, Quebec. Edited by R. Van Coillie, Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel and C. Thellen. Can. Tech. Rep. Fish. Aquat. Sci. 1989: 331 p. (MLCN: 95-00001).

Proceedings of the Nineteenth Annual Aquatic Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore. Can. Tech. Rep. Fish. Aquat. Sci. 1942: 489 p. (MLCN: 94-02230).

Proceedings of the Eighteenth Annual Aquatic Workshop: September 30-October 3, 1991, Ottawa, Ontario. Edited by A.J. Niimi and M.C. Taylor. Can. Tech. Rep. Fish. Aquat. Sci. 1863: 381 p. (MLCN: 92-07541).

Proceedings of the Seventeenth Annual Aquatic Workshop: November 5-7, 1990, Vancouver, British Columbia. Edited by P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuck and W. Knapp. Can. Tech. Rep. Fish. Aquat. Sci. 1774: 1213 p. (MLCN: 91-06176).

Proceedings of the Fifteenth Annual Aquatic Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. Van Coillie, A.J. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p. (MLCN: 90-01805).

Proceedings of the Fourteenth Annual Aquatic Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A.J. Niimi and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607:201 p. (MLCN: 88-04587).

Proceedings of the Thirteenth Annual Aquatic Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p. (MLCN: 88-01709).

Proceedings of the Twelfth Annual Aquatic Workshop: November 5-8, 1985. Edited by G. Ozburn. Can. Tech. Rep. Fish. Aquat. Sci. 1462: 229 p. (MLCN: 86-5828).

Proceedings of the Eleventh Annual Aquatic Workshop: November 13-15, 1984. Edited by G. Geen and K.L. Woodward. Can. Tech. Rep. Fish. Aquat. Sci. 1480: 330 p. (MLCN: 87-1493).

Proceedings of the Tenth Annual Aquatic Workshop: November 7-10, 1983, Halifax, Nova Scotia. Edited by P.G. Wells and R.F. Addison. Can. Tech. Rep. Fish. Aquat. Sci. 1368:

475 p. (MLCN: 86-1103).

Proceedings of the Ninth Annual Aquatic Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mckay. Can. Tech. Rep. Fish. Aquat. Sci. 1163: 243 p. (MLCN: 84-3262).

Proceedings of the Eighth Annual Aquatic Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 255 p. (MLCN: 83-2515).

Proceedings of the Seventh Annual Aquatic Workshop: November 5-7, 1980, Montreal, Quebec. Edited by N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert and M. Speyer. Can. Tech. Rep. Fish. Aquat. Sci. 990: 519 p. (MLCN: 82-0070).

Proceedings of the Sixth Annual Aquatic Workshop: November 6-7, 1979, Winnipeg, Manitoba. Edited by J.F. Klaverkamp, S.L. Leonhard and K.E. Marshall. Can. Tech. Rep. Fish. Aquat. Sci. 975: 291 p. (MLCN: 81-1492).

Proceedings of the Fifth Annual Aquatic Workshop: November 7-9, 1978, Hamilton, Ontario. Edited by P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns and U. Borgmann. Fish. Mar. Ser. Tech. Rep. 862: 342 p. (MLCN: 80-4061).

Proceedings of the Fourth Annual Aquatic Workshop: November 8-10, 1977, Vancouver, British Columbia. Edited by J.C. Davis, G.L Greer and I.K. Burtwell. Fish. Mar. Ser. Tech. Rep. 818: 211 p. (MLCN: 80:4022).

Proceedings of the Third Annual Aquatic Workshop Held in Halifax, Nova Scotia, November 2-3, 1976. Edited by W.R. Parker, E. Pessah, P.G. Wells and G.F. Westlake. Environment Canada, Surveillance Rep. EPS-5-AR-77-1.

Proceedings of the Second Annual Aquatic Workshop, November 4-5, 1975, Rexdale, Ontario. Edited by G.R. Craig. Ontario Ministry of the Environment.

Compendium of Aquatic Toxicity Studies in Canada. 1974. Unpublished Report, Freshwater Institute, Winnipeg, Manitoba. 39 p. + appendices.